SULFUR AND WETLAND PLANT DIVERSITY:
CALCAREOUS RICH FENS AS MODEL SYSTEMS

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EFFECT OF SULFUR ON WETLAND PLANT DIVERSITY: CALCAREOUS RICH FENS AS MODEL SYSTEMS

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Plant diversity in groundwater-fed wetlands is typically extraordinarily high, yet the biogeochemical controls of this diversity are still incompletely understood. I hypothesized that plant community composition could be related to a combination of fine-scale and broad-scale variation in sulfide via direct phytotoxicity and indirect mediation of phosphorus release from iron, coupled with gradients in other chemical constituents such as calcium. I measured porewater chemistry and associated plant species composition at nine groundwater-fed wetlands (rich fens), including one rich fen in which I intensively sampled 400 locations to capture fine-scale heterogeneity. Porewater sulfate and calcium concentrations were higher at the intensively sampled fen overlying gypsum geology than at other rich fens. Sulfide was highly variable within and across fens, ranging over two orders of magnitude in many fens. Inversely related concentrations of sulfide and ferrous iron in porewater were consistent with tight chemical coupling but were not readily traceable to phosphorus availability. Spatial patterns of sulfide and ferrous iron were conserved across seasons, with sulfide peaking with temperature in summer and ferrous iron peaking in fall at intermediate temperature. I used the corrected Akaike information criterion (AICc) to select among competing models of toxin, nutrient, and mixed-chemistry influences on vegetation. In the intensively sampled fen, models with a negative sulfide parameter provided the best explanation of total plant cover, cover of the three most frequently occurring species, dicot species density, and plant height. Calcium and phosphorus

combined with sulfide to explain some plant responses, but phosphorus alone did not explain any plant responses at the fine scale. Sulfide had a limited relationship with vegetation at the regional scale, only secondarily explaining total plant cover after first accounting for site-to-site variability. Gamma diversity values for individual sites were a negative power function of within-site sulfide variability values, with average alpha diversity for each site dominating. Overall, results from this work confirmed the relationship of rich fen vegetation to calcium and suggested that direct sulfide toxicity was a persistent but more moderate than expected stress to rich fen plants, while indirect sulfide mobilization of phosphorus was less important to plants than sulfide toxicity.

BIOGRAPHICAL SKETCH

Sam Simkin studied Biology and received a B.A. from Earlham College in 1995. Following college graduation he worked as an SCA Resource Assistant at Yosemite National Park and as an AmeriCorps member at Everglades National Park. For his M.S. in Conservation Ecology and Sustainable Development at the University of Georgia, co-advised by Drs. William Michener and Robert Wyatt, he studied plant response to fire and soil disturbance in the longleaf pine savanna of southern Georgia, at the Joseph W. Jones Ecological Research Center at Ichauway. He worked for Drs. Kathleen Weathers and Gary Lovett at the Cary Institute of Ecosystem Studies for five years, providing field, lab, and GIS assistance for studies of landscape patterns of atmospheric deposition of nitrogen and sulfur in Acadia National Park, Great Smoky Mountains National Park, the Catskill Mountains, and other areas. He also assisted with studies of stream water chemistry as a function of land cover and disturbance history in Catskill Mountain watersheds, nitrogen cycling in Yellowstone National Park, and fog deposition in California coast redwood forest. After working at the Cary Institute, he turned his attention to wetlands for his Ph.D. at Cornell University. Sam is married to Claire Benton and together they are the proud parents of their amazing and inspiring daughter Lydia.

This work is dedicated to my parents Jane and Paul, who first encouraged my curiosity about the world around me, and to my wife Claire and daughter Lydia, who daily inspire me to try to make the small piece of the world that I inhabit at least a little bit better than I found it.

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CHAPTER 1

OVERVIEW OF SULFUR AND ITS POTENTIAL EFFECTS ON WETLAND PLANTS

Overview

The research presented in this dissertation is a contribution to a broad effort to link patterns of plant species distributions with geochemical gradients at multiple spatial scales. There is a long and productive history of predicting plant community composition from environmental gradients (Vitt 1994), yet comprehensive explanations of biodiversity hotspots and biological invasions are still elusive. For example, existing climate envelope models used in the field of biogeography (Roberts and Hamann 2012) to attempt to predict plant species distributions at regional and global scales based on readily available precipitation and temperature data sometimes come up short due to unexplained environmental variation. Likewise, increased attention by biogeochemists to linkages of popular elements such as carbon, nitrogen, and phosphorus with other elements such as sulfur, iron, and calcium has unveiled new biogeochemical complexity (Burgin et al. 2011), but the ecological significance of these biogeochemical linkages to plant species distributions is still incomplete. My central research question revolves around determining why plant species occur where they do, and the premise of this research is that answering this question requires an understanding of linked biogeochemical cycling processes and the scale at which each element has its greatest effect on plants. The research in this dissertation explores this interface between plant species distributions and geochemical gradients in wetland ecosystems, as detailed below.

Wetlands are attractive model ecosystems for research linking plant species distributions

with biogeochemistry. Plant diversity can be extraordinarily high in some wetland types, notably rich fens that are by definition groundwater dependent (Amon et al. 2002, Bedford and Godwin 2003). Rich fens are model systems for studies of plant-chemistry relationships since they have spatially heterogeneous but temporally relatively persistent chemical gradients, in contrast with other wetlands such as coastal marshes that chemically reset each day with the influx of each tide. Existing research indicates that biogeochemical transformations of groundwater discharged from nested groundwater flow systems contribute to plant species distributions in rich fens (Bailey 2006), but there is still some uncertainty about which chemical elements are most important in influencing plant species abundance at which spatial scale. Sulfur content in bedrock and surficial geological materials, and therefore in groundwater discharge, is highly variable in many regions (Dean and Johnson 1989). Sulfate delivered by groundwater is reduced to sulfide by microbes, and this microbially produced sulfide is highly toxic to plants but also releases iron-bound phosphate. I hypothesized that some plant species would be more sensitive to sulfide toxicity, while other plant species would be more responsive to increased phosphorus availability.

Direct effects of sulfur on plants

Plant growth requires that sufficient sulfur be assimilated for the synthesis of essential organic sulfur compounds. In the traditional pathway of sulfur assimilation developed through work with the terrestrial *Arabidopsis thaliana* model plant species, sulfur in sulfate form is taken up by roots under strict regulatory control, transported up the vascular tissue by sulfate transporters, and reduced to H_2S in the chloroplasts via a series of steps that require multiple enzymes and a substantial expenditure of energy (Droux 2004, Saito 2004). The H_2S is then used

to synthesize the amino acid cysteine and then the amino acid methionine and other organic sulfur compounds. In practice, however, plant assimilation of sulfur is not always as tidy as this story suggests, especially in wetland plants. For example, plant capacity to regulate sulfate uptake can be overwhelmed (Hawkesford and De Kok 2006), plants can take up sulfur directly in an organic form such as glutathione (Seegmüller and Rennenberg 2002, Tausz et al. 2004), and some plant species can take up sulfur in the form of hydrogen sulfide from the atmosphere (Durenkamp and De Kok 2004) or porewater (Herschbach et al. 2005) and synthesize it directly into cysteine (Durenkamp and De Kok 2004). Plant metabolism of environmental hydrogen sulfide contradicts the paradigm that hydrogen sulfide is always a plant toxin, as discussed below.

The direct effect of sulfur on wetland plants that is most widely recognized is excessive sulfur uptake through roots in the toxic form of hydrogen sulfide. In wetlands, a significant portion of porewater sulfur is in the form of hydrogen sulfide, and plant uptake of hydrogen sulfide is not thought to be regulated as tightly as sulfate uptake (Ernst 1990). Furthermore, hydrogen sulfide gas (H_2S) is thought to pass through permeable cell membranes easily (Bagarinao 1992), whereas the hydrogen sulfide ion (HS^-) diffuses across membranes more slowly (Julian and Arp 1992) or not at all due to its charge (Vismann 1996). Porewater speciation of sulfide into H_2S and HS^- depends on pH, with about 70% in the form of H_2S at pH 6.5 and only 10% in the form of H_2S at pH 8.9 (Vismann 1996). A primary biochemical mode of H_2S toxicity is that, like cyanide, it interferes with aerobic respiration by binding the terminal cytochrome c oxidase enzyme in the mitochondria (Bagarinao 1992). Therefore, plants that are unable to switch to an anaerobic form of respiration such as fermentation by alcohol dehydrogenase (ADH) are unable to produce sufficient energy to survive in sulfidic

environments (Maricle et al. 2006). Sulfide may even inhibit ADH enzyme activity (Ernst 1990). Like other stressors, there are threshold hydrogen sulfide concentrations at which plants stop growing or die (Riemenschneider et al. 2005). Adaptations to sulfide by sulfide-tolerant organisms include sulfide-resistant respiratory enzymes, symbiotic relationships with sulfide-oxidizing bacteria, immobilization of sulfide, and detoxification of sulfide by methylation and mitochondrial oxidation of sulfide to thiosulfate and sulfate (Bagarinao 1992). Plant species that have a lower tolerance for hydrogen sulfide will decline at the expense of more sulfide-tolerant species (Smolders et al. 2003, Seliskar et al. 2004). For example, *Phragmites australis* does not begin to show clear effects of porewater sulfide until 375 uM (Chambers et al. 1998), in contrast to *Stratiotes aloides*, which is affected by porewater sulfide of only 5 uM (Smolders and Roelofs 1996).

Some plant species not only tolerate hydrogen sulfide but actively assimilate it, yet the range of sulfide concentrations for which this assimilation is relevant is currently unknown. Atmospheric H_2S at 0.2 ul L^{-1} (6 uM) can be directly assimilated into cysteine by terrestrial *Allium cepa* L. (Durenkamp and De Kok 2004), but this approaches the upper limit of foliar exposure studies since the maximum atmospheric hydrogen sulfide concentration is only 0.1 ul L^{-1} (3uM) even in heavily polluted areas (De Kok et al. 2002). In contrast, wetland plants are exposed to porewater hydrogen sulfide concentrations that range from negligible to 580 uM or more (Chambers et al. 1998). Furthermore, it is not known whether root tissue exposed to porewater sulfide might use the same mechanisms of hydrogen sulfide assimilation that are used by foliar tissue exposed to atmospheric hydrogen sulfide. For example, roots may not have sufficient O-Acetylserine (OAS) reactant, O-Acetylserine (thiol) lyase (OASTL) enzymes, or energy to convert sulfide to cysteine. It is impossible to extrapolate foliar assimilation of

atmospheric H_2S to root assimilation of porewater H_2S by wetland plants, but it is possible to relate wetland plant species occurrence to a wide range of porewater H_2S concentrations in the field.

Wetland plant exposure to sulfide is highly dependent on the microbially-mediated reduction and oxidation reactions that characterize wetland soils. Oxygen is the most favorable electron acceptor, but in saturated wetland soils the poor solubility of oxygen in water combined with oxygen consumption quickly leads to oxygen depletion. After oxygen and nitrate are depleted then iron reduction and reduction of sulfate to hydrogen sulfide are energetically favored and typically take on increased importance. Dissimilatory reduction of sulfate and other oxidized sulfur compounds to hydrogen sulfide by sulfate-reducing microbes should proceed if there is adequate oxidized sulfur and labile carbon. The counter reaction of sulfide oxidation to sulfate and other oxidized sulfur compounds simultaneously occurs in wetlands via sulfide oxidizing bacteria and chemical oxidation by oxygen. In marshes the net balance of sulfate reduction and sulfide oxidation swings wildly over time as the water table rises and falls, with this temporal variability making it difficult to establish any spatial pattern of where wetland plants are most exposed to porewater sulfide and sulfate. In wetlands such as fens that have a more stable water table there are not such large fluctuations in the net balance of sulfate reduction and sulfide oxidation. The relatively stable spatial patterns of fen porewater sulfide and sulfate make fens a model system for relating porewater sulfur chemistry to plant species composition within a wetland.

Indirect effects of sulfur on phosphorus and metal availability to plants

Sulfur indirectly influences phosphorus availability to plants, primarily via oxidation and

reduction reactions that include iron. Although sulfate has the potential to directly desorb phosphate from exchange sites (Abdin et al. 2003), this has typically been less important than sulfide-mediated phosphate mobilization (Roden and Edmonds 1997). Specifically, sulfide contributes to phosphate mobilization by chemically reducing iron and by precipitating iron as iron sulfide, which is an important sink for reduced iron (Roden and Edmonds 1997). If there is a net shift of iron from oxidized forms (e.g., iron oxyhydroxides) to reduced forms then phosphate formerly bound to oxidized iron can be released (Caraco et al. 1989, Lucassen et al. 2004a). Wetland soils with a significant fraction of phosphorus in the form of iron-bound phosphate therefore have the potential to mobilize increased phosphate in response to increased sulfate reduction to sulfide.

Phosphate mobilization is important because some wetlands, notably rich fens and bogs, have plants that are either P-limited or co-limited by N and P (Bedford et al. 1999). When phosphate is mobilized in phosphorus-poor wetlands, phosphorus-demanding plant species can grow more quickly and competitively displace phosphorus-conserving plant species, thereby decreasing plant species richness. In fact, losses of rare wetland species are more commonly caused by phosphorus enrichment than by nitrogen enrichment (Wassen et al. 2005). However, when internal eutrophication arising from sulfide is more moderate, it can actually increase wetland plant diversity (Bailey 2006).

Sulfide precipitates metals out of solution, thereby limiting the availability of those metals to plants. Precipitation of iron out of solution by sulfide has important consequences for phosphorus cycling as discussed earlier, but precipitation of iron out of solution can be important in its own right since reduced iron can reach levels that are toxic to plants (van der Welle et al. 2006). Sulfide can also decrease availability of metals to the point where plants are deficient,

with effects that may extend to higher trophic levels. For example, white-tailed deer grazing on *Salix exigua* Nutt. with high sulfur content and low cupper content may develop a copper deficiency (McBride 2007). To understand the extent to which sulfide controls toxicity or deficiency of metals to plants, it is again advantageous to examine a gradient of sulfide.

The indirect effects of sulfide on plants through mobilization of phosphorus and immobilization of metals are influenced not only by sulfur supply but also by redox conditions and pH, as with the direct effects. Increases in the wetland water table in spring lead to a more reducing environment and more phosphate mobilized. Later in the season when the water table drops and produces a more oxidized environment there should be more immobilization of phosphate. The speciation of iron shifts from oxidized Fe(III) to reduced Fe(II) as pH decreases (A.P.H.A. 2005). Examining a wetland gradient of sulfur supply that is independent from wetland gradients of pH and seasonal water table fluctuations pinpoints the role of sulfur supply specifically, while constraining the confounding variables of pH and temporal redox variability.

Gradients of sulfur supply: rich fens as model systems

The largest contrast in sulfur supply is between coastal wetlands and freshwater wetlands, but there is also a large gradient of sulfur supply within freshwater wetlands. Coastal wetland ecosystems are exposed to very high sulfate concentrations of up to the 2,700 ppm (28 mM) found in seawater (Schlesinger 1991), and consequently sulfide helps structure plant community zonation in those systems (Chambers et al. 1998, Koch et al. 2007). In contrast, sulfur supply to freshwater ecosystems is more heterogeneous. Freshwater wetland ecosystems are exposed to regional and local gradients of anthropogenic atmospheric sulfur deposition (Baumgardner et al. 2002, Weathers et al. 2006) and/or sulfur-containing fertilizer applications (Abrol and Ahmad

2003), but they can also be exposed to a large gradient of geological sulfur sources. The relative importance of sulfur from atmospheric deposition, fertilizer, geological, and other sources depends on the hydrogeological setting of a wetland. Generalizations about the influence of sulfur supply on freshwater wetland ecosystems and plants that fail to take into account the predominant source of sulfur will be flawed.

Taking into account a wetland's setting in the hydrologic and geologic landscape (Godwin et al. 2002) constrains the possible range of sulfur supply and subsequent plant responses. The hydrologic inputs to wetlands are precipitation, surface water, and ground water. Ground water dominates hydrologic inputs to fens. Within fens, variations in chemical composition of bedrock and surficial geological deposits, together with variations in the degree of contact that ground water flowpaths have with those geological materials, provide a large hydrogeologic gradient of sulfur supply within fens that influences plants. Peatland pipes several cm in diameter and hundreds of meters long (Holden et al. 2009) serve as conduits through the peat matrix with much greater hydraulic conductivity and add further heterogeneity in sulfur inputs. Regional gradients of atmospheric sulfur deposition are overwhelmed by the hydrogeologic gradient of sulfur in many fen wetland settings.

Rich fens are model systems for studying the effects of a gradient of sulfur supply upon wetland plants. The hydrology of rich fens is dominated by mineral-rich ground water with high alkalinity (Vitt 1994, Bedford and Godwin 2003). Rich fens therefore have a circumneutral to basic pH that is distinct from acidic bogs and a relatively stable water table that is distinct from marshes and riparian wetlands with greatly fluctuating water tables. The constrained range of pH and redox conditions in fens reduces the variability in sulfur and iron speciation that those variables would otherwise contribute, making it possible to focus directly upon the magnitude of

sulfur supply. The supply of sulfur to rich fens is strongly influenced by bands of gypsum bedrock and overlying gypsic surficial material (Newland and Leighton 1910, Fisher et al. 1970), while black shales and associated pockets of pyritic surficial material are another feature that may contribute additional geological heterogeneity in some areas. As an example of the wide range of sulfur that can occur in calcareous rich fens, consider that in New York State water in gypsic fens can have sulfate concentrations greater than 6 mM while in calcitic fens water can have sulfate concentrations averaging only 0.1 mM (Boomer and Bedford, unpublished data), which covers a significant portion of the range between the extremes seen at bogs on the low end and coastal marshes at the high end of the gradient of sulfur supply.

The wide range of sulfate in the groundwater that feeds rich fens may contribute to the high diversity of plants that can be found there. Within the large number of plant species found in rich fens, there are likely to be some species that respond strongly to the direct effects of sulfide toxicity or sulfide assimilation, other species that respond strongly to the indirect effects of sulfide-mediated phosphate mobilization, and still others that respond only weakly to sulfur supply instead of other variables. These species-level variations in response may be reflected by shifts in plant community composition along gradients of sulfur supply. Therefore, although the commonly recognized gradients of water table fluctuation, pH, and nutrients (Vitt 1994) help distinguish rich fens from other types of wetlands, I suggest that within rich fens there is an additional gradient of sulfur supply that must be taken into account to more fully understand wetland plant community composition.

In fens there has been a thorough series of papers addressing the effect of sulfur upon wetland plants in the Netherlands (Roelofs 1991, Lamers et al. 1998, Lucassen et al. 2004a, Lucassen et al. 2004b, Smolders et al. 2006), but it is not clear to what extent those studies can

be generalized to other fens. How common is it for ground water and surface water chemistry to be heavily polluted by agricultural runoff and atmospheric deposition such that there are sulfate concentrations greater than 3 mM (Lamers et al. 1998)? Should we expect different plant responses in regions with less heavily manipulated hydrologic regimes and less extensive diking of land near sea level? Are declines of plant species like *Stratiotes aloides* L. in the Netherlands following introduction of high sulfate water (Smolders et al. 2003) common elsewhere in the world? The answers to these questions require additional research with additional plant species across a wider gradient of sulfur supply.

Summary

I studied the relationship of sulfur to wetland plants within rich fens. By selecting rich fens as a model ecosystem I constrained the confounding influences of variations in pH and redox conditions, while making use of the wide range of geological settings that these ground-water dominated wetlands occupy. This research was multi-scalar. At one scale, the research complemented previous work addressing regional variation in hydrogeologic setting (Godwin et al. 2002) by filling in gaps in the continuum of sulfur availability, while at another scale the research complemented finer-scale work addressing within-wetland hydrologic influences on porewater chemistry (Boomer and Bedford 2008). These two previous bodies of work both indicated that sulfur in porewater influenced wetland plant community composition. By addressing sulfur porewater chemistry at multiple scales, the research in this dissertation provided further insights into the role of sulfur in structuring wetland plant communities, including identifying which wetland plant species are best explained by sulfide porewater chemistry. Ultimately, this dissertation addressed the research question of whether wetland plant

species diversity and community composition is more closely related to sulfur or to other porewater chemistry constituents. If wetland plants can be related to sulfur porewater chemistry, are these relationships more consistent with direct sulfide toxicity, indirect sulfide mobilization of iron-bound phosphorus, or a combination of both direct and indirect relationships with sulfide?

REFERENCES

A.P.H.A. 2005. 3500 - Fe (IRON). Page 3.76 to 73.79 *in* A. D. Eaton, L. S. Clesceri, E. W. Rice, and A. E. Greenberg, editors. Standard methods for the examination of water & wastewater. American Public Health Association, Washington, DC.

Abdin, M. Z., A. Ahmad, N. Khan, I. Khan, A. Jamal, and M. Iqbal. 2003. Sulphur interaction with other nutrients. Pages 359-374 *in* Y. P. Abrol and A. Ahmad, editors. Sulphur in Plants. Kluwer, Dordrecht.

Abrol, Y. P. and A. Ahmad. 2003. Sulphur in Plants. Kluwer, Dordrecht.

Amon, J. P., C. A. Thompson, Q. J. Carpenter, and J. Miner. 2002. Temperate zone fens of the glaciated Midwestern USA. Wetlands 22:301-317.

Bagarinao, T. 1992. Sulfide as an environmental-factor and toxicant - Tolerance and adaptations in aquatic organisms. Aquatic Toxicology 24:21-62.

Bailey, K. M. 2006. Effects of ground-water flow on soil chemistry, nutrient availability and plant species distributions in four New York State fens. Ph.D. dissertation. Cornell University, Ithaca, NY.

Baumgardner, R. E., T. F. Lavery, C. M. Rogers, and S. S. Isil. 2002. Estimates of the atmospheric deposition of sulfur and nitrogen species: Clean Air Status and Trends Network, 1990-2000. Environmental Science & Technology 36:2614-2629.

Bedford, B. L. and K. S. Godwin. 2003. Fens of the United States: Distribution, characteristics, and scientific connection versus legal isolation. Wetlands 23:608-629.

Bedford, B. L., M. R. Walbridge, and A. Aldous. 1999. Patterns in nutrient availability and plant diversity of temperate North American wetlands. Ecology 80:2151-2169.

Boomer, K. M. B. and B. L. Bedford. 2008. Influence of nested groundwater systems on reduction-oxidation and alkalinity gradients with implications for plant nutrient availability in four New York fens. Journal of Hydrology 351:107-125.

Burgin, A. J., W. H. Yang, S. K. Hamilton, and W. L. Silver. 2011. Beyond carbon and nitrogen:

how the microbial energy economy couples elemental cycles in diverse ecosystems. Frontiers in Ecology and the Environment 9:44-52.

Caraco, N. F., J. J. Cole, and G. E. Likens. 1989. Evidence for sulfate-controlled phosphorus release from sediments of aquatic systems. Nature 341:316-318.

Chambers, R. M., T. J. Mozdzer, and J. C. Ambrose. 1998. Effects of salinity and sulfide on the distribution of Phragmites australis and Spartina alterniflora in a tidal saltmarsh. Aquatic Botany 62:161.

De Kok, L. J., C. E. E. Stuiver, S. Westerman, and I. Stulen. 2002. Chapter 10: Elevated levels of hydrogen sulfide in the plant environment: nutrient or toxin. Pages 201-219 *in* K. Omasa, H. Saji, S. Youssefian, and N. Kondo, editors. Air pollution and plant biotechnology: prospects for phytomonitoring and phytoremediation. Springer, Tokyo.

Dean, W. E. and K. S. Johnson. 1989. Anhydrite Deposits of the United States and Characteristics of Anhydrite Important for Storage of Radioactive Wastes: An inventory of the geologic, physical, hydrologic, and chemical characteristics of anhydrite deposits in the United States and evaluation of anhydrite as a possible medium for the storage of radioactive wastes. USGS Bulletin 1794. United States Government Printing Office, Denver, CO.

Droux, M. 2004. Sulfur assimilation and the role of sulfur in plant metabolism: a survey. Photosynthesis Research 79:331-348.

Durenkamp, M. and L. J. De Kok. 2004. Impact of pedospheric and atmospheric sulphur nutrition on sulphur metabolism of *Allium cepa* L., a species with a potential sink capacity for secondary sulphur compounds. Journal of Experimental Botany 55:1821-1830.

Ernst, W. H. O. 1990. Ecophysiology of plants in waterlogged and flooded environments. Aquatic Botany 38:73-90.

Fisher, D. W., Y. W. Isachsen, and L. V. Rickard. 1970. Geologic Map of New York State, 1970, consisting of 5 sheets: Niagara, Finger Lakes, Hudson-Mohawk, Adirondack, and Lower Hudson. New York State Geological Survey Map and Chart Series. New York State Museum / New York State Geological Survey, Albany.

Godwin, K. S., J. P. Shallenberger, D. J. Leopold, and B. L. Bedford. 2002. Linking landscape properties to local hydrogeologic gradients and plant species occurrence in minerotrophic

fens of New York State, USA: A Hydrogeologic Setting (HGS) framework. Wetlands 22:722-737.

Hawkesford, M. J. and L. J. De Kok. 2006. Managing sulphur metabolism in plants. Plant, Cell and Environment 29:382-395.

Herschbach, C., S. Mult, J. Kreuzwieser, and S. Kopriva. 2005. Influence of anoxia on whole plant sulphur nutrition of flooding-tolerant poplar (Populus tremula x P-alba). Plant, Cell and Environment 28:167-175.

Holden, J., R. P. Smart, P. J. Chapman, A. J. Baird, and M. F. Billett. 2009. The role of natural soil pipes in water and carbon transfer in and from peatlands. Pages 251-264 Carbon Cycling in Northern Peatlands. AGU, Washington, DC.

Julian, D. and A. J. Arp. 1992. Sulfide permeability in the marine invertebrate *Urechis Caupo*. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology 162:59-67.

Koch, M. S., S. Schopmeyer, C. Kyhn-Hansen, and C. J. Madden. 2007. Synergistic effects of high temperature and sulfide on tropical seagrass. Journal of Experimental Marine Biology and Ecology 341:91-101.

Lamers, L. P. M., H. B. M. Tomassen, and J. G. M. Roelofs. 1998. Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. Environmental Science & Technology 32:199-205.

Lucassen, E. C. H. E. T., A. J. P. Smolders, J. van de Crommenacker, and J. G. M. Roelofs. 2004a. Effects of stagnating sulphate-rich groundwater on the mobility of phosphate in freshwater wetlands: a field experiment. Archiv Fur Hydrobiologie 160:117-131.

Lucassen, E. C. H. E. T., A. J. P. Smolders, A. L. Van der Salm, and J. G. M. Roelofs. 2004b. High groundwater nitrate concentrations inhibit eutrophication of sulphate-rich freshwater wetlands. Biogeochemistry 67:249-267.

Maricle, B. R., J. J. Crosier, B. C. Bussiere, and R. W. Lee. 2006. Respiratory enzyme activities correlate with anoxia tolerance in salt marsh grasses. Journal of Experimental Marine Biology and Ecology 337:30-37.

McBride, M. B. 2007. Trace metals and sulfur in soils and forage of a chronic wasting disease

locus. Environmental Chemistry 4:134-139.

Newland, D. H. and H. Leighton. 1910. Gypsum deposits of New York. New York State Museum, Education Department Bulletin Museum Bulletin 143:1-94.

Riemenschneider, A., V. Nikiforova, R. Hoefgen, L. J. De Kok, and J. Papenbrock. 2005. Impact of elevated H_2S on metabolite levels, activity of enzymes and expression of genes involved in cysteine metabolism. Plant Physiology and Biochemistry 43:473-483.

Roberts, D. R. and A. Hamann. 2012. Predicting potential climate change impacts with bioclimate envelope models: a palaeoecological perspective. Global Ecology and Biogeography 21:121-133.

Roden, E. E. and J. W. Edmonds. 1997. Phosphate mobilization in iron-rich anaerobic sediments: Microbial Fe(III) oxide reduction versus iron-sulfide formation. Archiv Fur Hydrobiologie 139:347-378.

Roelofs, J. G. M. 1991. Inlet of alkaline river water into peaty lowlands - Effects on water-quality and *Stratiotes aloides* L stands. Aquatic Botany 39:267-293.

Saito, K. 2004. Sulfur assimilatory metabolism. The long and smelling road. Plant Physiology 136:2443-2450.

Schlesinger, W. H. 1991. Chapter 9: The Oceans. Pages 291-341 Biogeochemistry: an analysis of global change. Academic Press.

Seegmüller, S. and H. Rennenberg. 2002. Transport of organic sulfur and nitrogen in the roots of young mycorrhizal pedunculate oak trees (Quercus robur L.). Plant and Soil 242:291-297.

Seliskar, D. M., K. E. Smart, B. T. Higashikubo, and J. L. Gallagher. 2004. Seedling sulfide sensitivity among plant species colonizing Phragmites-infested wetlands. Wetlands 24:426-433.

Smolders, A. J. P., L. P. M. Lamers, C. den Hartog, and J. G. M. Roelofs. 2003. Mechanisms involved in the decline of Stratiotes aloides L. in The Netherlands: sulphate as a key variable. Hydrobiologia 506:603-610.

Smolders, A. J. P., L. P. M. Lamers, E. Lucassen, G. Van der Velde, and J. G. M. Roelofs. 2006.

Internal eutrophication: How it works and what to do about it - a review. Chemistry And Ecology 22:93-111.

Smolders, A. J. P. and J. G. M. Roelofs. 1996. The roles of internal iron hydroxide precipitation, sulphide toxicity and oxidizing ability in the survival of *Stratiotes aloides* roots at different iron concentrations in sediment pore water. New Phytologist 133:253-260.

Tausz, M., B. Pilch, H. Rennenberg, D. Grill, and C. Herschbach. 2004. Root uptake, transport, and metabolism of externally applied glutathione in *Phaseolus vulgaris* seedlings. Journal of Plant Physiology 161:347-349.

van der Welle, M. E. W., M. Cuppens, L. P. M. Lamers, and T. G. M. Roelofs. 2006. Detoxifying toxicants: Interactions between sulfide and iron toxicity in freshwater wetlands. Environmental Toxicology and Chemistry 25:1592-1597.

Vismann, B. 1996. Sulfide species and total sulfide toxicity in the shrimp Crangon crangon. Journal of Experimental Marine Biology and Ecology 204:141-154.

Vitt, D. H. 1994. An overview of factors that influence the development of Canadian peatlands. Memoirs of the Entomological Society of Canada:7-20.

Wassen, M. J., H. O. Venterink, E. D. Lapshina, and F. Tanneberger. 2005. Endangered plants persist under phosphorus limitation. Nature 437:547-550.

Weathers, K. C., S. M. Simkin, G. M. Lovett, and S. E. Lindberg. 2006. Empirical modeling of atmospheric deposition in mountainous landscapes. Ecological Applications 16:1590-1607.

CHAPTER 2

MULTI-SCALE HETEROGENEITY IN CALCIUM, SULFUR, AND PLANT SPECIES COMPOSITION IN RICH FENS

Abstract

Many wetland environmental gradients structure plant community composition, yet controls of plant community composition within botanically diverse groundwater-fed wetlands are still incompletely understood. Porewater chemistry and plant community composition was recorded for nine calcareous rich fens in Central New York State. Heterogeneity in porewater sulfide and Fe(II), and to a lesser extent more commonly measured calcium, sulfate, nitrogen and phosphorus, was very high within and across groundwater-fed rich fens. Regional variation in porewater sulfate and calcium did not conform as cleanly to available geological and soil maps as expected. Porewater chemistry alone provided a poor explanation of the cover of plant functional groups and species in rich fens. Plant cover patterns related to sulfide, calcium and phosphorus emerged after site identity was explicitly included in models, suggesting that the modeled porewater chemistry variables only partially captured site-specific variations due to hydrogeologic setting and disturbance history. Species density at the plot scale was explained in part by sulfide and calcium. Species counts per fen declined as a negative power function of sulfide variability. In conclusion, rich fen wetland plant diversity per site decreased with increasing porewater sulfide variability. Porewater calcium, phosphorus, and sulfide partially explained plant cover, with the relative importance varying across plant functional groups and

species. Relative importance of porewater chemistry in influencing vegetation was sensitive to whether or not variability in unmeasured factors across fens was incorporated.

Introduction

Wetland plant species composition is strongly influenced by several environmental gradients, including hydrologic regime, water origin, base cations, salinity, pH, and nutrient availability (Vitt 1994), yet none of these gradients, alone or in combination, fully explains plant heterogeneity. For example, predominantly groundwater-fen wetlands, known as rich fens, typically have an abundance of ions as a direct consequence of receiving water mostly from belowground rather than from surface water or precipitation inputs (Amon et al. 2002, Bedford and Godwin 2003), limited nutrients (Boomer and Bedford 2008), and little or no salinity. Rich fens also normally have limited variability in water table elevations, although there are notable exceptions (Duval and Waddington 2011). Despite the environmental constraints outlined above, plant diversity in fens is proportionately much higher than their spatial extent would suggest. For example, in New York State 7% of the rare flora can be found in fens even though fens are only 0.07% of New York State's area (Bedford and Godwin 2003). In short, rich fens are wetlands with high conservation value that are model systems for studying the relationship of water chemistry to plant diversity.

A gradient of groundwater influxes to rich fens produces a corresponding gradient of ionic inputs reflecting the chemistry of the underlying soil and geological parent material. In some portions of the world the parent material is calcareous, including for example some study

sites in the Northeastern United States (Bedford and Godwin 2003), the upper Midwestern United States (Amon et al. 2002), the Netherlands (Lucassen et al. 2004b), Switzerland (Bergamini et al. 2009), and Slovakia (Horsak et al. 2012). Calcareous rich fens are known to support a subset of rich fen specialist calcicoles, but it is not entirely known whether the relationship of these calcicoles to plant community composition takes a binomial or linear form. Less recognized, but potentially important in some localities, are sulfur-rich parent materials such as gypsum ($CaSO_4 \cdot 2H_20$) (Newland and Leighton 1910, Fisher et al. 1970, Dean and Johnson 1989), black shales, and associated pockets of pyritic surficial material. Collectively, water chemistry constituents such as sulfur and calcium vary widely as a result of the underlying hydrogeologic heterogeneity.

The wide range of inputs of calcium, sulfur, and other elements to ground-water fed wetlands with differing hydrogeologic settings and associated underlying mineralogy and rates of groundwater movement has great potential to influence rich fen plant communities. An extensive literature already links calcium and calcium-loving plants (Clarkson 1965, White and Broadley 2003). Unresolved to date is the combined role of calcium and sulfur in plant community composition. The reduced sulfide form of sulfur is receiving increasing attention as a master variable influencing plants, not only through direct sulfide toxicity but also through indirect mobilization of iron-bound phosphorus by sulfide (Lamers et al. 1998, Boomer and Bedford 2008). Hydrogen sulfide is toxic since, like cyanide, it interferes with aerobic respiration in the mitochondria (Bagarinao 1992), thereby decreasing energy production and eliminating intolerant species. Sulfide releases phosphate formerly bound to oxidized iron by chemically reducing oxidized iron and precipitating that iron into the important iron sulfide sink for reduced iron (Caraco et al. 1989, Roden and Edmonds 1997, Lucassen et al. 2004a). In wetlands such as

rich fens and bogs that are phosphorus-limited or co-limited by nitrogen and phosphorus (Bedford et al. 1999) any sulfide-mediated phosphate mobilization could allow phosphorus-demanding plant species to competitively displace phosphorus-conserving plant species and decrease plant species richness.

Among the large number of plant species found in rich fens, there are likely to be some species that respond strongly to the direct effects of sulfide toxicity, other species that respond strongly to the indirect effects of sulfide-mediated phosphate mobilization, and still others that avoid or assimilate sulfide and therefore respond only weakly to sulfur instead of calcium and other variables. Furthermore, I expected that negative influences of sulfide on some plant species would be exploited by sulfide-intolerant species and that the positive influence of sulfide-mediated phosphate release on some plant species would displace slower-growing species. Collectively, I expected that the direct and indirect effects of sulfide on plants, combined with the impacts of those effects on inter-specific competition, would be reflected in shifts in plant species abundance and plant community composition along gradients of sulfur supply. Furthermore, I expected that these direct and indirect effects of sulfide might explain some variation in plant species composition formerly attributed to calcium alone.

The present study sought to identify whether there is a relationship of porewater sulfide to plant community composition that resolves some of the residual noise that would otherwise be left behind by the classic calcicole hypothesis. In particular I sought to examine plant community composition with respect to geological parent material across sites and with respect to reduced chemical products such as sulfide within sites to ascertain whether there are new linkages to be discovered between porewater chemistry and plant species abundance and composition. Specifically, I hypothesized that direct sulfide toxicity (Model 1 in Table 2-1), indirect sulfide

Table 2-1. Competing ecological hypotheses linking plant response to environmental variable(s). The general form of each equation is $\hat{y} = a + b*X + \varepsilon_{i,}$, where $\hat{y}$ is the predicted value of a plant response variable, a is the intercept, X is the first environmental predictor variable, b is the parameter estimate of variable X, and ε_i is the normally distributed residual error. Each of the 11 models are applied separately to each of the following plant response variables ($\hat{y}$): percent cover of each plant species, percent cover of each plant functional group, species density, plant height, percent bare ground.

Hypothesis ID	Hypothesis Description	Model/Equation
1) HS	Sulfide toxicity	$\hat{y} = a + b*HS + \varepsilon_{i,}$ where HS= single toxin sulfide
2) P	Phosphorus released to plants	$\hat{y} = a + b*P + \varepsilon_{i,}$ where P=single nutrient phosphorus
3) HS_P	Linked sulfide toxicity and phosphorus nutrient release	$\hat{y} = a + b*HS + c*P + \varepsilon_i$ where HS=sulfide and P=phosphorus
4) HS_CA	Linked sulfide toxicity and groundwater discharge proxy	$\hat{y} = a + b*HS + c*CA + \varepsilon_i$ where HS=sulfide and CA=calcium
5) CA	Groundwater discharge proxy or calcicole	$\hat{y} = a + b*Ca + \varepsilon_{i,}$ where CA=calcium
6) FE2	Iron toxicity or alternate index of phosphorus release	$\hat{y} = a + b*FE2 + \varepsilon_{i,}$ where FE2=single toxin Fe(II)
7) FE2_SO4	Phosphorus release and desorption	$\hat{y} = a + b*FE2 + c*SO4 + \varepsilon_i$ where FE2=Fe(II) and SO4=SO_4^{2-}
8) SO4_N	Atmospheric deposition	$\hat{y} = a + b*SO4 + c*N + \varepsilon_i$ where SO4=SO_4^{2-} and N=TDN
9) N_P	Nitrogen and phosphorus both available	$\hat{y} = a + b*N + c*P + \varepsilon_i$ where N=TDN and P=phosphorus
10) CA_FE2_SO4_N	Anything but sulfide toxicity or phosphorus release	$\hat{y} = a + b*CA + c*FE2 + d*SO4 + e*N + \varepsilon_i$
11) mean	Measured environmental variables not related to plant response variable.	$\hat{y} = \bar{y} + \varepsilon_i$ where $\bar{y}$ =mean of plant response

mobilization of phosphorus (Model 2 in Table 2-1), or a mixture of toxic and nutrient effects (Model 3 in Table 2-1) are better explanations of plant community composition than the classic calcicole explanation (Model 5 in Table 2-1) or other alternative explanations that can be put forth for rich fens (Models 6-11 in Table 2-1).

Methods

STUDY SITES

Sampling took place within nine calcareous rich fens (Figure 2-1) in central New York State, USA, that were expected to represent a broad gradient of sulfur and calcium supply based on previous research (Godwin et al. 2002, Boomer and Bedford 2008) and knowledge of hydrogeological setting. A summary of the nine focal fens, including surficial and bedrock geology, as well as soil survey descriptions, is displayed in Table 2-2. I established 10 sampling locations at each fen. These locations were co-located with previous sampling locations (Godwin et al. 2002, Boomer and Bedford 2008) wherever possible. At McLean Preserve Fen I added ten extra sampling locations to separately address the possibility of nutrient enrichment from an adjacent agricultural field (Drexler and Bedford 2002) that was cultivated as recently as three years before samples were collected for the present study. At Junius Pond Fen, for comparative purposes, I subsampled 10 out of the 400 locations previously sampled in a separate, spatially intensive, study (Chapter 4), selecting sites in a transect that paralleled a known groundwater flowpath (Godwin et al. 2002, Boomer and Bedford 2008). Two of the fens, Fish Fen and Mrs.

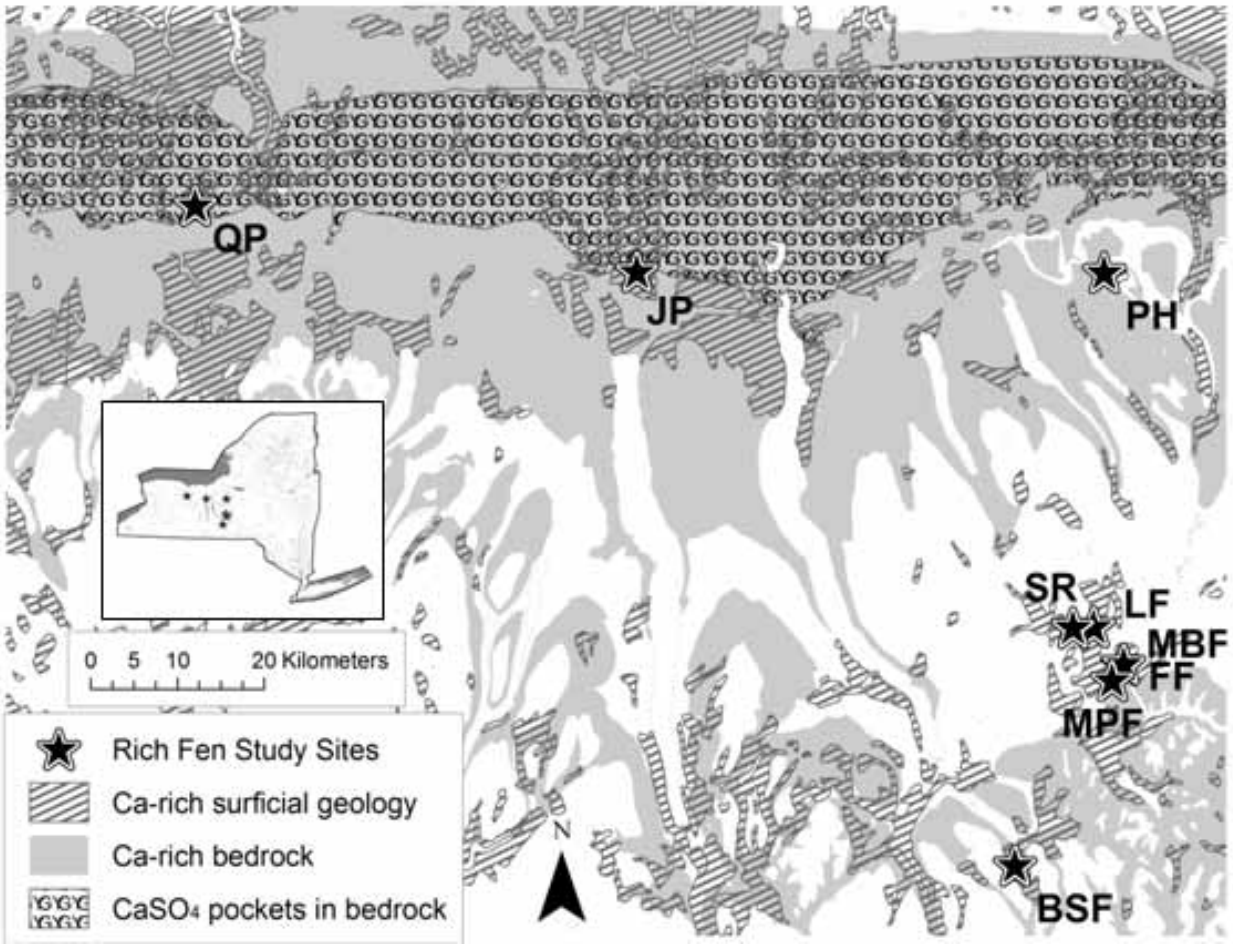


Figure 2-1. Site map of rich fen study site locations in the Finger Lakes region of New York State, with background showing several relevant calcium and sulfur geological features. Small inset map shows site locations within the border of New York State, USA.

Table 2-2. Characteristics of the 9 rich fen study sites. [1] fen type from the New York Natural Heritage Program(NYNHP) database (Olivero 2001). [2] fen not in NYNHP database but classified comparably. [3] Bedrock geology from (Fisher et al. 1970, Isachsen et al. 2000). [4] Surficial geology from (Cadwell et al. 1991). [5] Parent material group description from pmgroupname field in copmgrp table of (Soil Survey Staff 2012). [6] Soil horizon depth (The distance from the top of the soil to the base of the soil horizon) from hzdepb_r field in chorizon table of (Soil Survey Staff 2012).

Fen name	ID	previous chemistry sampling	fen type	N	[3] Bedrock age and bedrock geology	[4] Surficial geology	[5] Parent material group description	[6] Soil horizon depth
Quaker Pond	QP	Bedford et al. (1999)	[1] rich graminoid	10	Late Silurian – pockets of anhydrite and salt in dolomite and shale; Camillus Shale (incl. Salina Group)	peat-muck; 2-20 m thick	Mr = organic material	152 cm
Junius Pond	JP	Godwin et al (2002); Boomer and Bedford (2008)	[1]marl/rich, graminoid/ rich, shrub	400	Late Silurian – pockets of anhydrite and salt in dolomite and shale; Syracuse Formation & Akron Dolostone (incl. Salina Group)	kame moraine with **calcareous** cement; 10-30m thick	Mr = organic material	168 cm
Pumpkin Hollow	PH	Godwin et al (2002); Boomer and Bedford (2008)	[1] rich sloping	10	Early Devonian – sandstone and adjacent limestone and dolomite; Oriskany Sandstone (Onondaga Limestone and Tristates Group)	outwash sand and gravel; 2-20m thick	Ce = deep organic material	251 cm
Larry's Fen	LF	Crowley and Bedford (2011)	[1] rich sloping	10	Late Devonian – shale and sandstone; West River Shale (Genesee Group and Tully Limestone)	kame moraine with **calcareous** cement; 10-30m thick	EcA = loamy till dominated by siltstone, sandstone, and shale fragments	20 cm
Salt Road Fen	SR		[2] rich sloping	10	Late Devonian – shale and sandstone; West River Shale (Genesee Group and Tully Limestone)	till; 1-50m thick	Ws = silty and clayey alluvium washed from uplands that contain some **calcareous** drift AND EcA = loamy till dominated by siltstone, sandstone, and shale fragments	20-23 cm
Mrs. Baker's Fen	MBF	Bedford et al. (1999)	[1] rich sloping	10	Late Devonian – shale and sandstone; West River Shale (Genesee Group and Tully Limestone)	kame moraine with **calcareous** cement; 10-30m thick	SbA = loamy alluvium	23 cm
Fish Fen	FF	Godwin et al (2002); Crowley and Bedford (2011)	[1] rich sloping	10	Late Devonian – shale and sandstone; West River Shale (Genesee Group and Tully Limestone)	kame moraine with **calcareous** cement; 10-30m thick	Mc = organic material	178 cm
McLean Preserve Fen	MPF	Drexler and Bedford (2002)	[2] rich graminoid	20	Late Devonian – shale and sandstone; West River Shale (Genesee Group and Tully Limestone)	kame moraine with **calcareous** cement; 10-30m thick	Hc = loamy glaciofluvial deposits over sandy and gravelly glaciofluvial deposits AND HpE = gravelly loamy glaciofluvial deposits over sandy and gravelly glaciofluvial deposits,	20-23 cm
Belle School Fen	BSF	Crowley and Bedford (2011)	[1] rich sloping	10	Late Devonian – shale and sandstone; Cashaqua Shale (Sonyea Group)	kame moraine with **calcareous** cement; 10-30m thick	Ws = silty and clayey alluvium washed from uplands that contain some **calcareous** drift AND Em = silty alluvium	23-25 cm

Baker's Fen, were only 75 m apart, but the other fens were separated by distances of 2-121 km. Within fens, sampling locations were separated by distances of 2-112 m.

Information on disturbance history is available for two of the study sites. Fish Fen was dammed and diked in the 1850's to be a mill pond, received sawdust from a sawmill until the mill closed in 1870, and then had its dike breached around 1900 -1910 (F. Robert Wesley, personal communication). In at least several cores at Fish Fen, 20-50 cm of peat overlay a 2-12 cm layer of undecomposed sawdust (F. Robert Wesley, personal communication). The aptly named Salt Road that passes as close as 50 m to the nearest Salt Road Fen sampling location was used by salt wagons traveling from Syracuse salt mines to Ithaca from the 1790's to about the 1850's when salt transport would have shifted to the barge canal (F. Robert Wesley, personal communication), potentially explaining the elevated sodium and chloride concentrations (Simkin et al., unpublished data) at this site. These sites were included in subsequent data analyses, but the disturbances noted here are examples of the kinds of factors that could confound patterns in the measured porewater chemistry at a regional scale.

FIELD

In summer 2009 at each of the 10 sampling locations within each of the nine sites I measured plant community composition, porewater chemistry, and depth of peat. I recorded percent cover of each vascular and bryophyte species, bare, litter, and open water (if applicable), and the height of the overall tallest species in a small 0.1 m x 0.1 m quadrat from 24 August – 9 September 2009, with the exception of the Junius site that was sampled 6–14 June 2009. In the center of each vegetation quadrat I installed a porewater sipper (outer diameter 1 cm) with a

sample screen extending from 8 to 12 cm below the soil surface, for an average sampling depth of 10 cm. I used a bulk interstitial porewater sipper since I sought to measure sulfide at a scale matching a reasonable fraction of a plant's rhizosphere.

I collected porewater from each sipper using a hand syringe equipped with an in-line 0.45 uM filter 13–16 September 2009, with the exception of the Junius site that was sampled 26 May – 1 June 2009. In the field, I placed subsamples in three 23 mL borosilicate glass scintillation vials. In one vial I immediately mixed a 11.5 mL sample with 11.5 mL of previously-added sulfide anti-oxidant buffer (SAOB), filling the vial to capacity to minimize headspace, and set aside this sample aside for later sulfide analysis by ion selective electrode (ISE) in the lab as soon as possible. The SAOB was composed of NaOH, EDTA, and ascorbic acid to stabilize sulfide as S^{2-} to avoid oxidation to sulfate or volatile loss as H_2S. In a second vial I mixed 15 mL of sample with 5 mL of Ferrozine and HEPES reagents (Whitmire and Hamilton 2008) for ferrous iron analysis in the lab (Lovley and Phillips 1987). In a third vial I collected sample for later lab analysis of calcium, sulfate, and Total Dissolved Nitrogen (TDN). An additional sample was used to measure field pH, conductivity, and temperature with a portable multimeter and then discarded. Finally, I deployed a pair of 2.5 cm by 5 cm anion resin strips (GE Water & Process Technologies, product number AR204SZRA) at each sampling location from 30 July – 14 August 2009 at a depth of 1-3 cm and retrieved the strips 31 August – 16 September 2009 for an index of phosphorus availability. As with the other measurements, the Junius resin deployment was earlier, from 10–11 May 2009 to 8–12 June 2009.

LAB ANALYSES

I measured porewater sulfide as S^{2-} with an ion selective electrode (ISE) using sodium sulfide standards calibrated by lead perchlorate and then speciated sulfide into H_2S and HS^- following (A.P.H.A. 2005) and porewater Fe(II) (ferrous iron) spectrophotometrically at an absorbance of 562 nm (Stookey 1970, Lovley and Phillips 1987). Aqueous porewater samples were submitted to the Analytical Lab of the Cary Institute of Ecosystem Studies for inductively coupled plasma atomic-emission spectrometer (ICP-AES) analysis of calcium, ion chromatographic (IC) analysis of sulfate, and continuous-flow analysis (CFA) of Total Dissolved Nitrogen (TDN). Resin strips were extracted with 0.5 M HCl (Crowley and Bedford 2011) and then the resin extract samples were reacted with Murphy-Riley reagents (sulfuric acid, ammonium molybdate, antimony potassium tartrate) and analyzed spectrophotometrically for phosphate at 880 nm (Murphy and Riley 1962).

STATISTICAL ANALYSIS

In order to evaluate the relative strength of evidence for competing ecological hypotheses (Table 2-1) addressing the influence of environmental variables on cover and species richness of major plant functional groups, I used R (version 2.14) software to calculate the log likelihood and complexity of each model using the corrected Akaike information criterion (AICc). Models with the lowest AICc values were taken to be the best fit given the data available. In addition to cover and species richness, I used the AIC framework to look at competing models explaining plant height, and percent bare ground as a function of environmental variables.

Unlike plant functional groups, individual plant species percent cover values had zero-inflated distributions that could not be normalized, so I used a two-part modeling approach in

which the first component of the model evaluates presence versus absence in a binomial distribution of the generalized linear model (GLM), while the second component of the model looks at square-root transformed cover values at only those locations where the species is present. I summed the maximum likelihoods of the two parts of the model and conservatively used the lower sample size of the cover analysis in the second part of the model to calculate AICc information criteria values. Subsequently for each species I identified the model with the least information loss (lowest AICc value) and subtracted that from the AICc value of the other ten models to calculate the ΔAICc of each model. The model set (Table 2-1) was balanced with regard to the number of times that each of the six environmental values (sulfide, phosphorus, calcium, Fe(II), sulfate, and nitrogen) occurred in a model, permitting calculation of model weights from ΔAICc (Anderson 2008) values as follows: model weight =exp(-ΔAICc/2)/ sum(exp(-ΔAICc/2))). The sum of the model weights totals one for each species, and an individual model with a higher model weight has a better fit to the model, given the data. Finally, I used model weights to calculate average parameter estimates, given the entire model set (Anderson 2008). Note that model weights alone do not give the magnitude or direction of influence of component environmental variables. This latter piece of information can only be obtained from the weighted parameter estimates.

In addition to the model runs described above for continuous porewater chemistry variables only, those same models were all re-run after including a categorical site variable specifying the fen name. This alternate model set runs the risk of overparamaterization and loss of generality, but the inclusion of a site variable provides the benefit of potentially providing some clarity by capturing some unmeasured and confounding factors such as anthropogenic effects and history, hydraulic conductivity, landscape position, length of groundwater flowpath,

and mineralogy that are specific to each fen. If models with the site variable added provide a better explanation, then it suggests that the porewater chemistry variables that I examined were insufficient on their own to either directly influence plant response or indirectly serve as an index of underlying components of the hydrogeologic setting.

Plant diversity measures were calculated as indicated below. Gamma diversity values were calculated for each of the nine sites and were considered to be the total number of plant species recorded at the ten sampling locations within each site. We did not address the additional level of diversity encompassing the total species in all nine sites, what could be termed delta diversity (Whittaker 1977), even though that is the scale semantically implied in the usage of gamma diversity by many authors (Anderson et al. 2011). Alpha diversity values for each of the nine sites were considered to be the average of the species richness values for the ten sampling locations within each site. Beta diversity was calculated as gamma diversity divided by alpha diversity.

In order to visualize variation in plant community composition and relate it to environmental gradients I ordinated plots in plant species space using nonmetric multidimensional scaling (NMS), a multivariate analysis that is still robust when there are many zero values, as is common with plant community data (McCune and Grace 2002). I implemented NMS with function metaMDS of the R package vegan (Oksanen et al. 2011), using Bray-Curtis/Sorensen distances, a starting configuration derived from metric scaling, 200 data runs, and three dimensions. I removed species occurring in less than five of the nine sampled rich fen sites (McCune and Grace 2002) or in less than 7% of the sampled quadrats, and the remaining species cover values were square-root transformed. Axes scores were centered, rotated to align with PC axes, and halfchange scaled. I sought a convergent solution in 200 runs, and

failing that I used the solution with the lowest stress as the final solution. I added scaled species correlations with the first two NMS axes and calculated the linear correlations of those axes with the following environmental variables: total sulfide (H_2S plus HS^-), resin phosphorus, calcium, ferrous iron, sulfate, total dissolved nitrogen, temperature corrected conductivity, and pH.

Results

POREWATER CHEMISTRY WITH RESPECT TO GEOLOGY

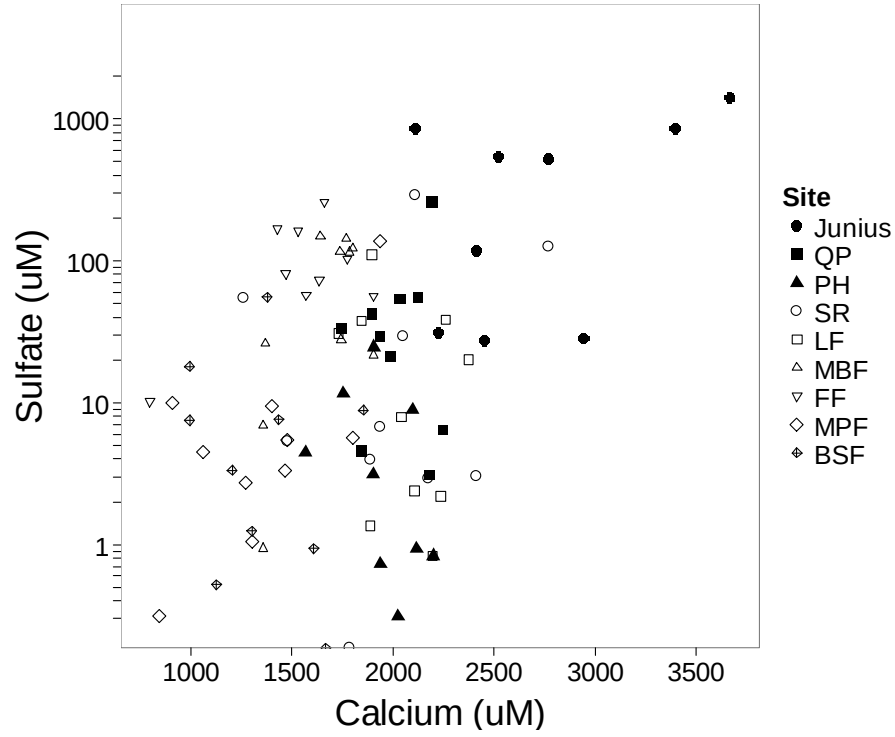
Porewater sulfate ranged from 0-1405 uM (mean=87) across the nine studied fens. I expected that fens occurring where statewide maps showed underlying gypsum bedrock (Fisher et al. 1970, Isachsen et al. 2000) and shallow surficial geology (Cadwell et al. 1991) (Table 2-2, Figure 2-1) would have the highest porewater sulfate concentrations, but this was only partially supported by the data. Junius Pond (JP) fen had dramatically higher sulfate than anywhere else and straddled the border between gypsum and non-gypsum bedrock, with 10-30 m of intervening kame moraine, making it the one fen whose sulfate mapped cleanly with geological maps (Figure 2-2a). In contrast, the Quaker Pond (QP) fen situated over gypsum bedrock with just 2-20 m of intervening surficial peat muck had only one high sulfate location and was otherwise comparable or even lower in sulfate than sites overlying nominally calcitic geology. Likewise, sulfate at Pumpkin Hollow (PH) fen was low even though PH was just south of the gypsum zone and therefore appeared that it would be marginally influenced by gypsum. Conversely, two neighboring fens separated by only 75 m that were both nominally overlying the same sulfur-

poor calcitic geology had distinctly different sulfate concentrations, with sulfate typically relatively high at Fish Fen (FF), but the neighboring Mrs. Baker's Fen (MBF) having bimodal sulfate concentrations that were sometimes higher than the FF site and sometimes lower.

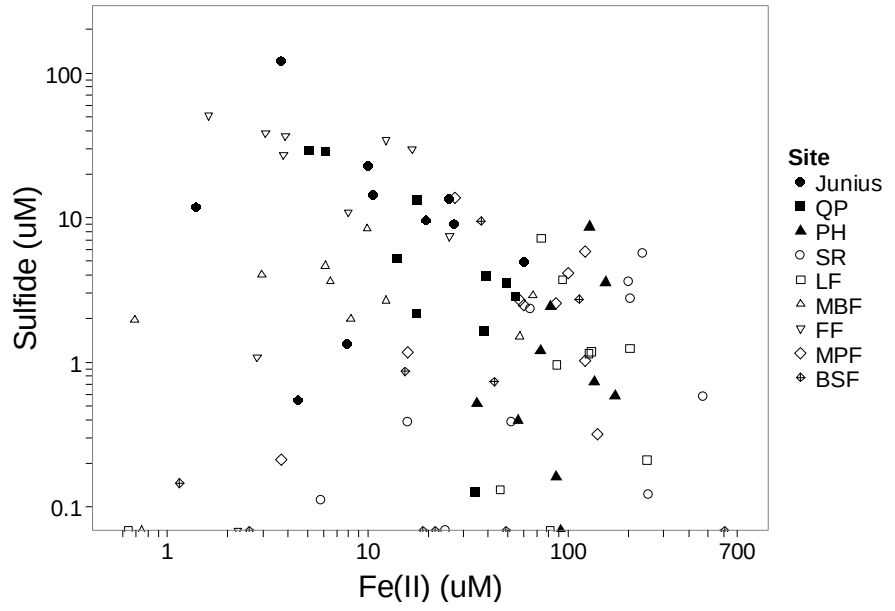
Porewater calcium ranged from 798-3,668 uM (mean=1,838). Calcium was ostensibly missing from surficial geology only at Salt Road (SR) and Pumpkin Hollow (PH) based on maps (Table 2-2), yet these two fens were in the upper tier of high calcium fens (Figure 2-2a). The most noticeable trend in porewater calcium was that it was higher in the northernmost sites (QP, JP, and PH) than in the four southernmost sites (MBF, FF, MPF, and BSF) (Figure 2-2a). However, SR and LF were only slightly north of MBF and FF and yet were more comparable to the two northern sites (Figure 2-1).

Porewater sulfide ranged from 0-121 uM (mean=7). As with sulfate, sulfide conformance to state level geology maps of gypsum was mixed. The two fens mapped on gypsum bedrock (JP and QP) included locations high in porewater sulfide, especially JP whose upper limit of sulfide was an order of magnitude higher than most other fens (Figure 2-2b). However, porewater sulfide was also high at the fen (FF) not ostensibly situated over gypsum bedrock in which hydrology was modified by dikes and peat was disturbed by the addition of sawdust in the sawmill era. In contrast with the high sulfide at FF, sulfide at MBF only ~ 75 m away was comparable to other sites (Figure 2-2b), so something besides broad-scale geological or climatic variation was clearly occurring, perhaps connected to previous disturbances of FF noted in the study site descriptions.

Porewater ferrous iron, phosphorus, and nitrogen did not match geological map units. The three fens with the highest sulfide (JP, QP, and FF) had low Fe(II) (Figure 2-2b), consistent with expectations that sulfide and Fe(II) would precipitate each other out of solution. Fe(II) at SR



(a)



(b)

Figure 2-2 (a) Porewater sulfate versus calcium. (b) Porewater sulfide versus Fe(II). Sample size is 10 plots for each of nine sites.

fen was somewhat bimodal, and BSF had one outlying location with Fe(II) that was the highest measured in this study (Figure 2-2b). Overall, Fe(II) ranged from 0.6-604 uM (mean=66). Resin phosphorus ranged from 2-478 umoles mo^{-1} $10cm^{-2}$, except for an outlying high value of 2,814 at QP. Total dissolved nitrogen ranged from 11-74 uM, except for an outlying high value of 153 at BSF.

VEGETATION CHARACTERISTICS

Plant species cover was high for moss and lower for dicots and monocots, while plant species density (species per $100cm^2$ plot) of mosses, monocots and dicots were variable but generally high. Moss cover averaged 55% but was as high as 88% at MBF and as low as 19% or 25% at SR and PH, while average species density was highest at FF (2.7) and MBF (2.6) (Figure 2-3a). Monocot cover averaged 13% but was as high as 34% at SR and as low as 4% at MPF and FF, while average species density averaged as high as 3.3 at LF and as low as 0.3 at FF (Figure 2-3b). Dicot cover averaged 18% and was relatively even across sites except QP which averaged 6%, while species density averaged as high as 3.1 at MPF and as low as 0.7 at QP (Figure 2-3c).

A large proportion of the 90 plant species sampled in this study were specialists (Table 2-3). Specifically, 13 species were characteristic marl specialists (Olivero 2001) and 57 species were characteristic specialists of marl fens or other rich fens (Olivero 2001), and more than 90% were characteristic wetland specialists. Only 23 of 73 vascular plant species were typically found in forested or open uplands in addition to wetlands (Wesley et al. 2008). One species, *Scleria verticillata*, is an endangered species in New York State. An additional four vascular plant species (*Morella penslyvanica*, *Carex sterilis*, *Cladium mariscoides*, and *Eleocharis rostellata*)

Table 2-3. Annotated species list indicating degree of habitat selectivity and regional abundance. [1]Data on characteristic (c) or occasional (o) occurrence in marl or rich fens extracted from (Olivero 2001). [2]Data on general habitat occurrence and regional abundance from (Wesley et al. 2008). The names of the species that occurred most frequently in this study are highlighted in bold.

species	Vegetation Type	[1]marl fen	[1]Any marl or rich fen	[2]Any Wetland	[2]Upland (**F**orest or **O**pen)	[2]Freq. in Cayuga Basin	[2]Native or Introduced
Acer rubrum	dicot		c	W	FO	Common	Native
Achillea	dicot				O	Common	Introduced
Asclepias incarnata	dicot			W		Frequent	Native
Chelone glabra	dicot		o	W		Common	Native
Dasiphora floribunda	dicot	c	c	W		Scarce	Native
Doellingeria umbellata	dicot		c	W		Frequent	Native
Drosera rotundifolia	dicot		c	W		Frequent	Native
Epilobium leptophyllum	dicot			W		Frequent	Native
Eupatoriadelphus maculatus	dicot		c	W		Common	Native
Euthamia graminifolia	dicot		**c**		O	Common	Native
Fragaria virginiana	dicot		**c**		FO	Common	Native
Galium labradoricum	dicot			W		Scarce	Native
Galium palustre	dicot			W		Frequent	Native
Geum rivale	dicot		c	W		Frequent	Native
Hydrocotyle americana	dicot			W		Frequent	Native
Impatiens capensis	dicot			W		Common	Native
Lobelia kalmii	dicot	c	c	W		Scarce	Native
Lobelia siphilitica	dicot			W		Common	Native
Lycopus uniflorus	dicot	c	c	W	O	Common	Native
Lysimachia ciliata	dicot			W	F	Common	Native
Mentha arvensis	dicot		c	W	O	Frequent	Introduced
Mentha spicata	dicot			W	O	Common	Introduced
Mentha x piperita	dicot			W	O	Common	Introduced
Morella penslyvanica	dicot		c	W	F	Rare	Native
Packera aurea	dicot		c	W		Frequent	Native
Parnassia glauca	dicot	c	c	W		Scarce	Native
Pilea pumila	dicot			W	F	Common	Native

Ranunculus acris	dicot				O	Common	Introduced
Rhamnus alnifolia	dicot		c	W		Frequent	Native
Rubus pubescens	dicot		c	W	F	Frequent	Native
Salix discolor	dicot		**c**	W		Common	Native
Sarracenia purpurea	dicot	c	c	W		Scarce	Native
Solidago patula	dicot		c	W		Frequent	Native
Solidago rugosa	dicot				FO	Common	Native
Solidago uliginosa	dicot		c	W		Scarce	Native
Symphotrichum puniceum	dicot		**c**	W		Common	Native
Taraxacum officinale	dicot				O	Common	Introduced
Thalictrum pubescens	dicot		c	W	F	Frequent	Native
Vaccinium macrocarpon	dicot		**c**	W		Scarce	Native
Dryopteris cristata	fern			W		Frequent	Native
Thelypteris palustris	fern		c	W		Common	Native
Thuja occidentalis	gymno.	c	c	W	FO	Scarce	Native
Equisetum arvense	horsetail		c	W	FO	Common	Native
Equisetum fluviatile	horsetail		**c**	W		Frequent	Native
Agrostis stolonifera	monocot			W	O	Common	Introduced
Calamagrostis canadensis	monocot		**c**	W		Common	Native
Carex aquatilis	monocot		c	W		Scarce	Native
Carex flava	monocot	c	c	W		Frequent	Native
Carex hystericina	monocot		c	W		Frequent	Native
Carex lacustris	monocot			W		Frequent	Native
Carex lasiocarpa	monocot		d	W		Frequent	Native
Carex leptalea	monocot		c	W		Frequent	Native
Carex prairea	monocot		c	W		Scarce	Native
Carex sterilis	monocot		c	W		Rare	Native
Carex stricta	monocot		c	W		Frequent	Native
Cladium mariscoides	monocot	c	o	W		Rare	Native
Dichanthelium acuminatum	monocot			W	O	Frequent	Native
Eleocharis elliptica	monocot			W		Scarce	Native
Eleocharis rostellata	monocot	c	c	W		Rare	Native
Glyceria canadensis	monocot			W		Scarce	Native
Glyceria striata	monocot		**c**	W		Common	Native
Iris versicolor	monocot		c	W		Common	Native

Juncus brevicaudatus	monocot			W		Scarce	Native
Muhlenbergia glomerata	monocot		c	W	O	Scarce	Native
Phalaris arundinacea	monocot			W	O	Common	Native
Phragmites australis	monocot			W		Common	Introduced
Platanthera psycodes	monocot			W		Frequent	Native
Poa pratensis	monocot			W	O	Common	Native
Scirpus acutus / Schoenoplectus acutus	monocot	c	c	W		Scarce	Native
Scleria verticillata	monocot	**c**	c	W		Rare	Native
Symplocarpus foetidus	monocot		c	W		Common	Native
Typha angustifolia	monocot		c	W		Frequent	Native
Typha latifolia	monocot		c	W		Common	Native
Aneura pinguis	misc_nv		c				
Chara	misc_nv	**c**	c				
Aulacomnium palustre	moss						
Bryum pseudotriquetrum	moss		c				
Calliergonella cuspidata	moss		c				
Campylium stellatum	moss	c	c				
Fissidens adianthoides	moss		c				
Plagiomnium ellipticum	moss						
Plagiothecium laetum	moss						
Scorpidium scorpioides	moss		o				
Sphagnum centrale	moss						
Sphagnum squarrosum	moss						
Sphagnum teres	moss		o				
Sphagnum warnstorfii	moss		c				
Thelia hirtella	moss						
Thuidium delicatulum	moss		c				
Tomentypnum nitens	moss						

are rare in the Cayuga Lake Basin and 15 more vascular plant species are scarce in the Cayuga Lake Basin (Wesley et al. 2008). Of the six most frequently occurring species in this study, one is rare (*Carex sterilis*) and two are scarce (*Solidago uliginosa* and *Muhlenbergia glomerata*) within the Cayuga Lake Basin (Wesley et al. 2008). In summary then, even the most abundant species in this study of rich fens are often quite uncommon in the landscape at large.

MODEL COMPARISONS OF VEGETATION IN RELATION TO POREWATER CHEMISTRY

Sulfide, calcium, or phosphorus explained the percent cover of several specific plant species but did not explain the percent cover of plant functional groups. The model that best explained the monocot cover, moss cover, total live cover, and bare cover was the "anything but sulfide or phosphorus" model (10 in Table 2-1), and the model that best explained the dicot cover was the mean model (11) that included just the average cover and no environmental variables (Table 2-4). At the individual species level the mean model was often best too, with several notable exceptions. For *Solidago patula* and *Solidago uliginosa*, the sulfide-only model (1) with a negative parameter for the binomial component of *S. patula* and a negative parameter for the quantitative component of *S. uliginosa* was best (Table 2-4). For *Calliergonella cuspidata*, the calcium-only model (5) with negative calcium parameters was best. For *Packera aurea*, the phosphorus-only model (2) with a positive parameter in the quantitative cover component of the two-step model was best. In the only other plant responses with any substantial weight to the phosphorus parameters, *S. patula* and total dicot cover followed the same pattern as *P. aurea*. Similarly for calcium, in the only other plant responses with any substantial weight to

the calcium parameters, total moss cover followed the same pattern as *C. cuspidata*, and *S. uliginosa* had a negative parameter in only the binomial component of the model (Table 2-4).

Unlike plant cover, species density within plant functional groups may be explained in part by sulfide or calcium (Table 2-4). Moss species density was best explained by models with a negative calcium parameter. Dicot species density was best explained by models with negative calcium and sulfide parameters. Monocot species density was approximately equally well explained by the sulfide-only model with a negative sulfide parameter or by no environmental variables at all (mean model). Another notable plant response in addition to species density was plant height, which was best explained by models including a positive nitrogen parameter and negative phosphorus parameter (9) (Table 2-4).

The role of sulfide in plant diversity extends to measured gamma diversity of study sites. The gamma diversity, here defined as the cumulative total number of sampled species per fen, was a negative power function of the standard deviation of sulfide within each fen, with the sulfide deviation representing a measure of the number of different sulfide niches (Figure 2-4). Contrary to expectation, this gamma diversity was contained almost entirely within the alpha diversity rather than in the beta diversity (Fig 2-4).

CHANGES IN POREWATER CHEMISTRY MODELS WITH THE ADDITION OF A SITE VARIABLE

Plant cover patterns emerged with respect to porewater chemistry when a categorical site variable was added (Table 2-5). Overall cover was best explained by models including a negative calcium (5) parameter, either alone or in combination with a negative sulfide parameter (4),

coinciding with the same pattern in mosses. Consistent with the plant cover pattern, bare cover was best explained by models including a positive calcium parameter, either alone or in combination with a positive sulfide parameter. Monocot cover was approximately equally well explained by a negative phosphorus parameter or by no environmental variables at all.

After adding the site variable, species richness of mosses and dicots was best explained by the mean model (11) rather than by calcium-only (5) or sulfide plus calcium models, indicating that adding the site variable reduced the ability of porewater-based models to explain these plant responses. Adding the site variable shifted monocot species richness from being weakly supported by a sulfide-alone model (1) with a negative parameter to a more strongly supported iron-alone model (6) with a negative parameter.

MULTIVARIATE ANALYSES

The first NMS ordination axis was strongly correlated with calcium, indicating that vegetation community composition changed along a calcium gradient (Figure 2-4). Sulfate and conductivity were also correlated with the first axis (not shown), but not as strongly as calcium. Vegetation plots within the JP fen mostly clustered together on the high calcium end of the first NMS axis, but for other fens the within-fen variability was qualitatively similar to variability across fens (Figure 2-5). With the exception of the Junius site, sampling locations within the same site didn't cluster together on ordination axes. Phosphorus was primarily correlated with the second ordination axis (Figure 2-5). Sulfide was not an important part of the ordination but is displayed for illustrative purposes only (Figure 2-5). Nitrogen, Fe(II), and pH were the most poorly correlated with the ordination axes and are not displayed.

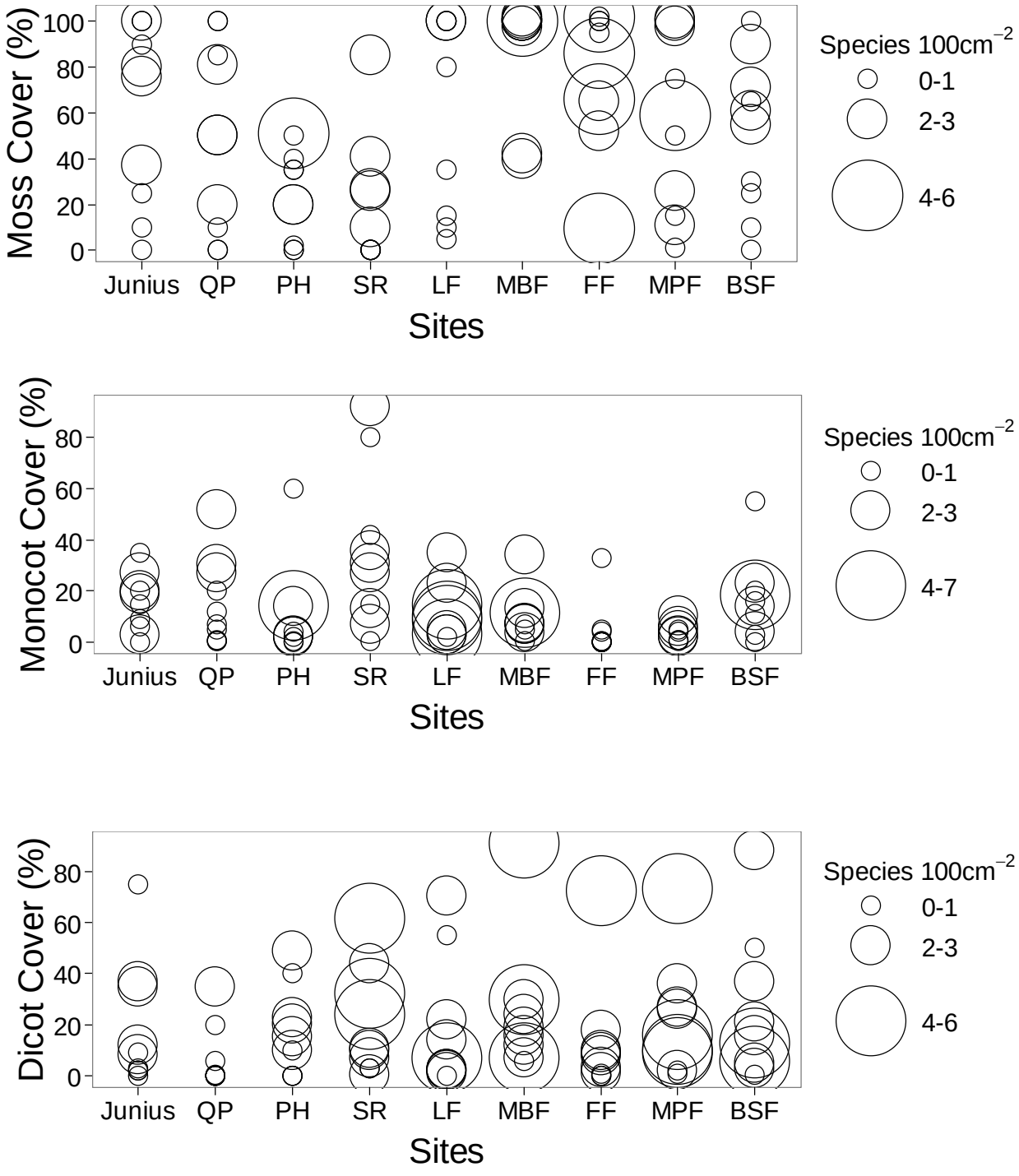


Figure 2-3. Plant cover of each functional group, with symbol size indicating species density.

Table 2-4. Model importance and parameters of vegetation models. Each row represents a single plant functional group cover, species cover, functional group richness, or other plant response, displayed in descending order according to frequency of occurrence. For each species or functional group, the hypothesis/model from Table 2-1 with the greatest weight is identified and its model weight is displayed as a decimal. The right-hand columns of the table are average parameter estimates, weighted by model weights. In the top half of the table, presenting cover results, only parameter estimates for the three variables (sulfide, phosphorus, and calcium) that were expected to be the most important *a priori* are presented, but separate parameter estimates for both parts of each model are provided: binomial (presence/absence only) and Gaussian (percent cover only where cover > 0). In the lower half of the table binomial models were not appropriate and instead parameter estimates of all six variables are presented. Negative parameter estimates are highlighted in bold.

		Best model	Importance of best model	HS (binom)	HS	P (binom)	P	CA (binom)	CA
Functional groups	Freq.								
TOTL cover	86	CA_FE2_SO4_N	0.75						**-20**
MONO cover	79	CA_FE2_SO4_N	0.99					54	1
DICO cover	77	mean	0.28					**-2**	**-2**
MOSS cover	76	CA_FE2_SO4_N	0.43					**-7**	**-9**
Species	Freq.								
CAST cover	47	mean	0.54						
SOPA cover	27	HS	0.75	**-12949**	704				
CACU cover	25	CA	0.78					**-9**	**-12**
MUGL cover	19	mean	0.79						
SOUL cover	15	HS	0.65	292	**-17137**				
ANPI cover	14	mean	0.76						
CAHY cover	14	mean	0.56						
CSTE cover	14	mean	0.80						
FIAD cover	14	mean	0.95						
PAAU cover	13	P	0.53			**0**	520		
Plant response				HS_wt	P_wt	CA_wt	FE2_wt	SO4_wt	N_wt
height (cm)		N_P	0.67		**-188**				7734
bare cover		CA_FE2_SO4_N	0.45			116	315	**-59**	2322
moss (spp. density)		CA	0.36			**-3**			
dico (spp. density)		HS_CA	0.40	**-1968**		**-5**			
mono (spp. density)		HS	0.16	**-800**					

Table 2-5. Model importance and parameters of vegetation models after adding a site variable. Cover models are in the top rows, species density and other plant responses are in the bottom rows. Table layout is the same as in Table 2-4.

	Best model	Importance of best model	HS (binom)	HS	P (binom)	P	CA (binom)	CA
Functional group								
TOTL cover	HS_CA	0.59		**-4259**				**-30**
MONO cover	P	0.31			**-98**	**-2**		
DICO cover	mean	0.78						
MOSS cover	CA	0.76					**-1**	**-30**
Plant response			HS_wt	P_wt	CA_wt	FE2_w	SO4_wt	N_wt
height (cm)	N_P	0.90		**-420**				7132
bare cover	HS_CA	0.66	70150		221			
moss (spp. density)	mean	0.31						
dico (spp. density)	mean	0.32						
mono (spp. density)	FE2	0.31				**-15**		

Species correlations with ordination axes confirmed the importance of calcium and downgraded the importance of some of the sulfide and phosphorus patterns from the univariate modeling analysis. *Dasiphora floribunda* and *Eleocharis rostellata* were positively correlated with the calcium axis (Figure 2-5). *Calliergonella cuspidata* was negatively correlated with the calcium axis (Figure 2-5), consistent with the univariate modeling analysis (Table 2-4). *Packera aurea* did not align with the phosphorus vector (Figure 2-5), which was not consistent with the univariate modeling analysis (Table 2-4).

Discussion

REGIONAL VARIABILITY IN POREWATER SULFATE

Porewater concentrations of sulfate in rich fens could not be reliably and consistently predicted from maps of underlying geology that are currently available for the region included in this study. Gypsum (calcium sulfate) mineralogy in this region has a heterogeneous distribution in pockets which have varied horizontal area and vertical depth (Newland and Leighton 1910) that can not be readily captured in coarse-scale maps. Black shale covers broad areas and under the right conditions can include pyrites (FeS_2) (Schieber and Baird 2001), but the spatial distribution of the most pyritic portions of these shales is not specified in state-wide geology maps. Topography, position in the landscape, depth of till, and other components of hydrogeologic setting beyond geological mineral composition influence the delivery of solutes to the surface of fens. Finally, present or historic disturbances may obscure a strong link between

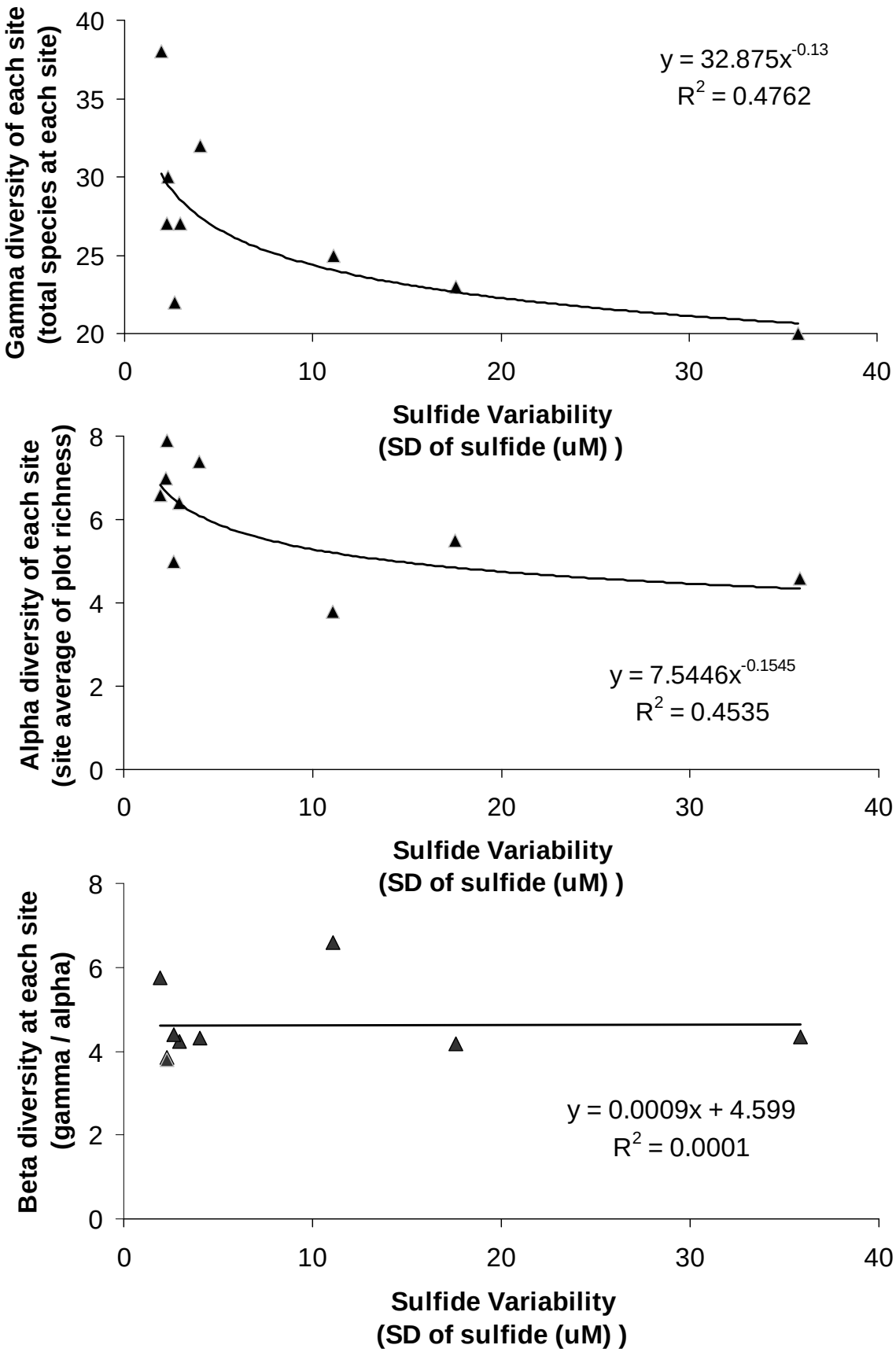


Figure 2-4. Diversity of a site as a function of sulfide variability.

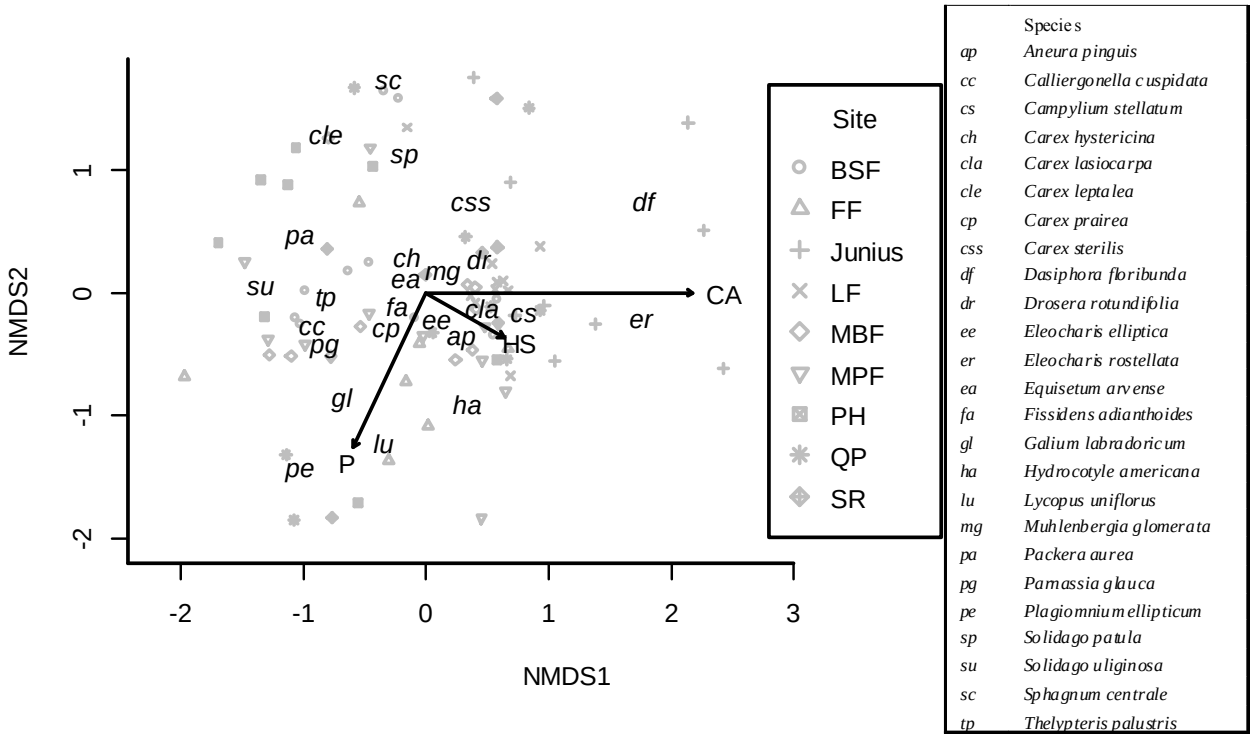


Figure 2-5. Non-metric multidimensional scaling (NMS) ordination of square-root transformed cover data of plots (gray symbols) in species space. Symbol shapes indicate the site in which a plot occurred. Species correlations with axes are shown as lowercase two character species codes in italics, with the first letter indicating the first letter of the genus and the second letter indicating the first letter of the species. Biplot vectors of environmental variables are shown in capitals at the end of solid black vector arrows: CA=calcium (uM), P= phosphorus (umol mo^{-1} $10cm^{-2}$), and HS= total (H_2S + HS^-) sulfide (uM).

porewater sulfur and geology. For example, during the sawmill era at Fish Fen the hydrology was modified by dikes and the peat was directly modified by sawdust additions, possibly overwhelming the signature of the underlying geology. Landscape patterns of atmospheric sulfur deposition (Weathers et al. 2006) were unlikely to have obscured geological patterns of sulfur inputs given the dominant role of ground-water in the water budget of rich fens. More detailed geological maps and knowledge of disturbance history would have likely improved the predictability of regional porewater sulfate trends.

The range of sulfate measured in this study (3-1,405 uM) encompassed the full range of known sulfate values for rich fens in this region, with the exception of two large outliers at Camillus (6,460 uM) (Godwin et al. 2002) and at Byron-Bergen Swamp (13,429 uM sulfate) (Simkin, unpublished data). Farther afield, average sulfate concentrations of 1,070 or 1,729 uM sulfate (Lucassen et al. 2005) were observed in some of the Netherlands fens where much research has been conducted linking sulfide toxicity and phosphorus release. Meanwhile, acid mine drainage can be 1,600 uM sulfate (Herlihy et al. 1988, as estimated from Holmer and Storkholm 2001) and ultimately seawater averages 28,232 uM sulfate (Schlesinger 1997, citing Holland 1978). Sulfate does not predict sulfide but it does constrain sulfide, and on this basis I can say that my study sites included a substantial portion of the possible non-tidal range of sulfur inputs.

CALCIUM RELATIONSHIP TO VEGETATION

Calcium was associated with vegetation at a regional scale even though it didn't span the low range below 900 uM and is not a strong toxin. Sulfide ranged from the very low range to

high range and is a strong toxin, yet was less closely associated with vegetation than expected. This suggests that either Ca is more physiologically active than I thought at very high concentrations, that sulfide is oxidized in the rhizosphere long before making contact with plant's internal tissues, both of the above, or some other factor.

SULFIDE AND PLANT DIVERSITY

The cumulative number of species sampled per fen decreased when sulfide was more variable, contrary to expectations. The *a priori* expectation had been that sulfide variability within fens would increase species pools at the whole fen scale by providing a diversity of niches for plants with divergent capacities to compete versus tolerate sulfide. Instead, plant species competitiveness and sulfide tolerance may have been more coupled than expected and sulfide variability may not have provided useful information beyond that provided by average sulfide. In this revised interpretation, sulfide may have simply decreased the number of species per fen by filtering out those species that were least tolerant of sulfide.

Sulfide had a limited influence on plant species density at the 0.01 cm^2 plot level, only partially explaining decreased monocot species density in combination with calcium. Furthermore, this role for sulfide disappeared after including a site variable that encompassed unmeasured differences among fens. This limited influence of sulfide on plant species density at the plot level was unexpected. Several possible causes for both this result and the limited sulfide influence on plant cover are discussed below.

SULFIDE AND PLANT COVER

The relatively weak response of individual plant species cover to sulfide was surprising at a theoretical level given the direct toxicity of sulfide and the indirect role of sulfide in mobilizing phosphorus, and was surprising empirically based on results from others (Lamers et al. 1998, Boomer and Bedford 2008) and a companion study (Chapter 4). In the present study only two species (*Solidago patula* and *Solidago uliginosa*) had a negative sulfide parameter in either presence-absence or quantitative cover components of the two-step models that were tested, and even these two species lacked negative sulfide parameters for both steps of the two-step models. Contrary to expectations, there apparently were not species with high root aeration, high sulfide assimilation capacity, or other sulfide tolerance or avoidance strategies thriving at the expense of sulfide-intolerant species. Instead, all species currently persisting in these fens apparently have some degree of sulfide tolerance. Alternatively, if the pattern of decreased total plant cover and increased bare cover with increased sulfide after accounting for unmeasured differences between sites is emphasized, then all species were slightly negatively impacted by sulfide, but no species more so than another.

WHY WASN'T THE INFLUENCE OF SULFIDE ON PLANT SPECIES DENSITY AND COVER STRONGER?

I did not experimentally manipulate sulfide in the field because of concern for the sensitivity of the sites, and therefore I cannot rule out the possibility that my modeling approach did not successfully disentangle sulfide from sulfate, calcium, ferrous iron, and other variables as I had intended. As an example, calcium to sulfate ratios ranged from 2-6,500, with a median of

89, which was beneficial in that these were not linear covariates but challenging in that an extremely large sample size would have been required to thoroughly blanket the range of possible combinations. For this reason, follow-up experimental mesocosm work with the two species (*Solidago patula* and *Solidago uliginosa*) that showed the greatest indication of a negative sulfide effect would certainly be warranted and fruitful.

Disturbance history is another confounding factor potentially explaining why the relationship between sulfide and plant responses was weaker than expected. The high sulfide observed in FF, the former mill pond with a layer of sawdust, contrasted greatly with the low sulfide in MBF just 75 m away. The contrast between these two sites was as big as any within the study even though geology, climate, atmospheric deposition, and almost any other imaginable underlying influence should have been nearly identical, pointing to the likelihood of an influence of disturbance in this instance and raising the possibility of other more subtle and unmeasured disturbance effects on other fens. If disturbances such as damming a mill pond and adding sawdust to it influenced only sulfide and the other porewater attributes measured in this study then I would have accounted for the disturbance. However, if the disturbance altered carbon quality or trace amounts of other unknown and unmeasured elements then this could have confounded any of the porewater chemistry explanatory variables that I explicitly considered.

Plant responses to sulfide may have been nonlinear and may have had a lower threshold than anticipated. Instead of a gradual decline in plant energy balance and subsequent growth rate as sulfide increased from 0.5 to 50 uM, all aerobic respiration may have already shut down at 0.1 uM sulfide or lower and any additional sulfide may have been superfluous. I did not measure rates of plant aerobic or anaerobic respiration to address this issue. I had anticipated that secondary aspects of sulfide exposure like sequestration of reduced sulfur as glutathione for

stress resistance would confer additional distinctions between sulfide tolerant and sulfide intolerant species beyond toxicity avoidance but I may have been wrong. Arguing against this interpretation that sulfide thresholds are very low, however, is the apparently wide range of sulfide sensitivity in salt marsh species (Chambers et al. 1998, Seliskar et al. 2004).

CHALLENGES AND INSIGHTS FROM SCALING PLANT RESPONSES TO GEOCHEMISTRY FROM LOCAL TO REGIONAL SCALES

Plant-environment relationships in this regional (>1,000 km^2) study differed from a separate study (Chapter 4) at a local (<0.0016 km^2) scale, illustrating the risk of assuming that results can simply be extrapolated from one scale to another. In contrast with the local study (Chapter 4), the best models of cover for plant functional groups and species in this regional study often did not include sulfide or other porewater variable unless differences between sites were taken into account. One interpretation that would reconcile these results is that sulfide and other porewater chemistry influences plant cover variation around a mean within a single fen, but that mean plant cover at a site is determined by some other factor such as disturbance history.

Moss cover was one example of a plant response in which sulfide was a consistently negative influence at both regional and local scales, but the role of another contributing water chemistry parameter varied. Specifically, calcium was a negative influence in this regional study but was in contrast a positive influence in a separate local study (Chapter 4). Knowing that calcium concentrations were higher in the single fen than in the other regional fens, this suggests that it is moderate calcium concentrations that suppress plant growth. One explanation may be that that in lower calcium fens most of the plant species are calcifuges that decline with

increasing calcium while in the single higher calcium fen many of the plant species are calcicoles that thrive when calcium increases.

Plant height was an alternative plant response to cover that helped accommodate the response of certain sedges and other narrow-leaved plants with an upright stature, and so the shift in explanatory variables from local (<0.0016 km^2) to regional (>1,000 km^2) scales was intriguing. At the local scale sulfide and calcium provided the best explanation (Chapter 4), while in contrast nitrogen and phosphorus provided the best explanation in the present regional study. The combination of the strongly positive role of nitrogen and the weakly negative role of phosphorus in influencing plant height in this regional study was puzzling as fens are usually not thought to be strictly nitrogen limited. It is also puzzling that plant height and bare cover did not have an inverse relationship with porewater chemistry in the present regional study as they did in the intensive study (Chapter 4). A simple explanation for the discrepancy between the spatial scales is not readily apparent, highlighting the potential risks of extrapolating results from one spatial scale to another.

SELECTED SPECIES RESPONSES THAT WERE SURPRISING OR NEGLECTED

Species generally thought to be phosphorus demanding, such as *Typha latifolia*, occurred infrequently in this study. This was partially a sampling artifact of the small plot size used and *Typha*'s upright growth habit, but perhaps also indicates that the range of phosphorus measured in this study was below the threshold at which a noticeable plant response would be expected. The one plant species with some indication of a positive response to phosphorus, *Packera aurea*, deserves further research as it is typically a minor component of the plant community and does

not fit the usual profile of a phosphorus-demanding species.

Tissue sulfur concentrations recorded elsewhere suggested that *Solidago patula* might be able to regulate sulfate uptake (Simkin, unpublished data) reasonably well, yet models including sulfate did not explain *S. patula* abundance. This could be interpreted as meaning that sulfate uptake can be regulated with relatively low energetic cost and therefore no discernible influence of sulfate on cover can be observed.

A number of other classic rich fen indicator species, such as *Eupatorium maculatum*, which had previously shown some indication of weak capacity to regulate sulfate uptake (Simkin, unpublished data), occurred too infrequently to be analyzed, highlighting the challenge of understanding ecological controls of rarer species that are visually eye-catching and perhaps of greater societal interest, but are difficult to assess unless specifically targeted for sampling.

SUMMARY

Overall, at a regional scale the abundance of a few species was well-explained by porewater sulfide (*Solidago patula* and *Solidago uliginosa*), calcium (*Calliergonella cuspidata*), or phosphorus (*Packera aurea*), but other plant species and functional groups were relatively poorly explained by porewater chemistry. Results elsewhere supporting the inclusion of sulfur as an additional environmental gradient structuring wetland plant community structure at a sub-hectare scale (Chapter 4) must apparently be qualified by certain as yet unidentified site-specific factors. The role of calcium and sulfide in explaining cover of plant functional groups was contingent on including a non-generalizable categorical site variable. In contrast, the same site variable removed the explanatory role of sulfide and calcium with regard to species density of

mosses and dicots, and shifted monocot species density from a model weakly supported by sulfide to a model more strongly supported by iron. This raises the possibility that there may be multiple unmeasured confounding differences among sites. The factors that explain species density may covary with calcium and sulfide, while the factors that explain plant cover might not covary with calcium and sulfide. Unmeasured site-specific factors may have set floors and ceilings of plant cover, yet species richness for some reason may have been less obscured by those same site-specific factors. The weaker relationship between sulfide and plant response in this regional study compared to an intensive study of 400 sampling locations in a single fen (Chapter 4) illustrates the challenge of attempting to scale results from a fine scale to a broad scale. However, the importance of working out species-environment relationship across spatial scales despite the challenges is illustrated by the emergence of a negative relationship between species pool size and sulfide variability across fens.

REFERENCES

A.P.H.A. 2005. 4500 - S^{2-} (SULFIDE). Page 4.170 to 174.183 *in* A. D. Eaton, L. S. Clesceri, E. W. Rice, and A. E. Greenberg, editors. Standard methods for the examination of water & wastewater. American Public Health Association, Washington, DC.

Amon, J. P., C. A. Thompson, Q. J. Carpenter, and J. Miner. 2002. Temperate zone fens of the glaciated Midwestern USA. Wetlands 22:301-317.

Anderson, D. R. 2008. Model based inference in the life sciences: a primer on evidence. Springer, New York.

Anderson, M. J., T. O. Crist, J. M. Chase, M. Vellend, B. D. Inouye, A. L. Freestone, N. J. Sanders, H. V. Cornell, L. S. Comita, K. F. Davies, S. P. Harrison, N. J. B. Kraft, J. C. Stegen, and N. G. Swenson. 2011. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecology Letters 14:19-28.

Bagarinao, T. 1992. Sulfide as an environmental-factor and toxicant - Tolerance and adaptations in aquatic organisms. Aquatic Toxicology 24:21-62.

Bedford, B. L. and K. S. Godwin. 2003. Fens of the United States: Distribution, characteristics, and scientific connection versus legal isolation. Wetlands 23:608-629.

Bedford, B. L., M. R. Walbridge, and A. Aldous. 1999. Patterns in nutrient availability and plant diversity of temperate North American wetlands. Ecology 80:2151-2169.

Bergamini, A., M. Peintinger, S. Fakheran, H. Moradi, B. Schmid, and J. Joshi. 2009. Loss of habitat specialists despite conservation management in fen remnants 1995–2006. Perspectives in Plant Ecology, Evolution and Systematics 11:65-79.

Boomer, K. M. B. and B. L. Bedford. 2008. Influence of nested groundwater systems on reduction-oxidation and alkalinity gradients with implications for plant nutrient availability in four New York fens. Journal of Hydrology 351:107-125.

Cadwell, D. H., G. G. Connally, P. J. Fleisher, E. H. Muller, R. A. Young, R. J. Dineen, J. L. Rich, M. L. Fuller, L. Sirkin, G. C. Wiles, D. L. Pair, D. A. Franzi, J. T. Gurrieri, G. M. Haselton, G. C. Kelley, R. G. LaFleur, and J. S. Street. 1991. Surficial Geologic Map of

New York consisting of five sheets: Finger Lakes, Hudson-Mohawk, Niagara, Lower Hudson and Adirondack. New York State Geological Survey Map and Chart Series. New York State Museum / New York State Geological Survey, Albany.

Caraco, N. F., J. J. Cole, and G. E. Likens. 1989. Evidence for sulfate-controlled phosphorus release from sediments of aquatic systems. Nature 341:316-318.

Chambers, R. M., T. J. Mozdzer, and J. C. Ambrose. 1998. Effects of salinity and sulfide on the distribution of Phragmites australis and Spartina alterniflora in a tidal saltmarsh. Aquatic Botany 62:161.

Clarkson, D. T. 1965. Calcium uptake by calcicole and calcifuge Species in the genus *Agrostis* L. Journal of Ecology 53:427-435.

Crowley, K. and B. Bedford. 2011. Mosses influence phosphorus cycling in rich fens by driving redox conditions in shallow soils. Oecologia 167:253-264.

Dean, W. E. and K. S. Johnson. 1989. Anhydrite Deposits of the United States and Characteristics of Anhydrite Important for Storage of Radioactive Wastes: An inventory of the geologic, physical, hydrologic, and chemical characteristics of anhydrite deposits in the United States and evaluation of anhydrite as a possible medium for the storage of radioactive wastes. USGS Bulletin 1794. United States Government Printing Office, Denver, CO.

Drexler, J. Z. and B. L. Bedford. 2002. Pathways of nutrient loading and impacts on plant diversity in a New York peatland. Wetlands 22:263-281.

Duval, T. P. and J. M. Waddington. 2011. Extreme variability of water table dynamics in temperate calcareous fens: Implications for biodiversity. Hydrological Processes 25:3790-3802.

Fisher, D. W., Y. W. Isachsen, and L. V. Rickard. 1970. Geologic Map of New York State, 1970, consisting of 5 sheets: Niagara, Finger Lakes, Hudson-Mohawk, Adirondack, and Lower Hudson. New York State Geological Survey Map and Chart Series. New York State Museum / New York State Geological Survey, Albany.

Godwin, K. S., J. P. Shallenberger, D. J. Leopold, and B. L. Bedford. 2002. Linking landscape properties to local hydrogeologic gradients and plant species occurrence in minerotrophic fens of New York State, USA: A Hydrogeologic Setting (HGS) framework. Wetlands 22:722-737.

Horsak, M., M. Hajek, D. Spitale, P. Hajkova, D. Dite, and J. C. Nekola. 2012. The age of island-like habitats impacts habitat specialist species richness. Ecology 93:1106-1114.

Isachsen, Y. W., E. Landing, J. M. Lauber, L. V. Rickard, and W. B. Rogers. 2000. Geology of New York: a simplified account. Second Edition edition. New York State Museum / Geological Survey, State Education Dept., University of the State of New York, Albany, NY.

Lamers, L. P. M., H. B. M. Tomassen, and J. G. M. Roelofs. 1998. Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. Environmental Science & Technology 32:199-205.

Lovley, D. R. and E. J. P. Phillips. 1987. Rapid Assay For Microbially Reducible Ferric Iron In Aquatic Sediments. Applied and Environmental Microbiology 53:1536-1540.

Lucassen, E., A. Smolders, L. Lamers, and J. Roelofs. 2005. Water table fluctuations and groundwater supply are important in preventing phosphate-eutrophication in sulphate-rich fens: Consequences for wetland restoration. Plant and Soil 269:109-115.

Lucassen, E. C. H. E. T., A. J. P. Smolders, J. van de Crommenacker, and J. G. M. Roelofs. 2004a. Effects of stagnating sulphate-rich groundwater on the mobility of phosphate in freshwater wetlands: a field experiment. Archiv Fur Hydrobiologie 160:117-131.

Lucassen, E. C. H. E. T., A. J. P. Smolders, A. L. Van der Salm, and J. G. M. Roelofs. 2004b. High groundwater nitrate concentrations inhibit eutrophication of sulphate-rich freshwater wetlands. Biogeochemistry 67:249-267.

McCune, B. and J. B. Grace. 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, OR.

Murphy, J. and J. P. Riley. 1962. A Modified Single Solution Method For Determination Of Phosphate In Natural Waters. Analytica Chimica Acta 26:31-36.

Newland, D. H. and H. Leighton. 1910. Gypsum deposits of New York. New York State Museum, Education Department Bulletin Museum Bulletin 143:1-94.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2011. vegan: Community Ecology Package. R package version 2.0-2. http://CRAN.R-project.org/package=vegan.

Olivero, A. M. 2001. Classification and Mapping of New York's Calcareous Fen Communities. New York Natural Heritage Program, Albany, NY.

Roden, E. E. and J. W. Edmonds. 1997. Phosphate mobilization in iron-rich anaerobic sediments: Microbial Fe(III) oxide reduction versus iron-sulfide formation. Archiv Fur Hydrobiologie 139:347-378.

Schieber, J. and G. Baird. 2001. On the origin and significance of pyrite spheres in Devonian black shales of North America. Journal of Sedimentary Research 71:155-166.

Seliskar, D. M., K. E. Smart, B. T. Higashikubo, and J. L. Gallagher. 2004. Seedling sulfide sensitivity among plant species colonizing Phragmites-infested wetlands. Wetlands 24:426-433.

Soil Survey Staff. 2012. Soil Survey Geographic (SSURGO) Database. Natural Resources Conservation Service, U.S. Dept. of Agriculture. http://soildatamart.nrcs.usda.gov/ Accessed [June 2012].

Stookey, L. L. 1970. Ferrozine - A New Spectrophotometric Reagent For Iron. Analytical Chemistry 42:779-781.

Vitt, D. H. 1994. An overview of factors that influence the development of Canadian peatlands. Memoirs of the Entomological Society of Canada:7-20.

Weathers, K. C., S. M. Simkin, G. M. Lovett, and S. E. Lindberg. 2006. Empirical modeling of atmospheric deposition in mountainous landscapes. Ecological Applications 16:1590-1607.

Wesley, F. R., S. Gardescu, and P. L. Marks. 2008. Vascular Plant Species of the Cayuga Region of New York State. Cornell University online publications: Species List, Ithaca, NY. 63 pages. http://hdl.handle.net/1813/9413:63.

White, P. J. and M. R. Broadley. 2003. Calcium in plants. Annals of Botany 92:487-511.

Whitmire, S. L. and S. K. Hamilton. 2008. Rates of anaerobic microbial metabolism in wetlands of divergent hydrology on a glacial landscape. Wetlands 28:703-714.

Whittaker, R. H. 1977. Evolution of species diversity in land communities. Evolutionary Biology

10:1-67.

CHAPTER 3

A PERSISTENT SPATIAL PATTERN OF PHYTOTOXIC SULFIDE AND FERROUS IRON ACROSS SEASONS IN A TEMPERATE GROUNDWATER-FED WETLAND

Abstract

Wetland porewater chemistry can exhibit high spatial heterogeneity in the warm season, but it is largely unknown whether this heterogeneity persists year-round. No seasonal shifts in the spatial patterns of reduced chemical species such as sulfide and Fe(II) (ferrous iron) should be expected if the controls of rates of abiotic and biotic oxidation and reduction are semi-permanently fixed in space. Spatial patterns of sulfide and ferrous iron could persist even if absolute concentrations declined with system-wide shifts such as temperature. Conversely, if redox controls are transient or mobile then spatial patterns could shift seasonally, and if system-wide shifts were sufficiently extreme to shut down reduction then any spatial pattern could be almost entirely removed. In order to determine whether spatial patterns of sulfide and ferrous iron are conserved year-round or instead shift or disappear seasonally I intensively measured porewater sulfide and Fe(II) concentrations at 400 locations within a calcareous rich fen in summer, fall and winter seasons. Seasonal persistence of spatial patterns of sulfide and Fe(II) accumulation was indicated by positive correlations between summer, fall, and winter concentrations even though absolute concentrations decreased with temperature in winter. Winter declines in reduced chemicals were accompanied by only modest decreases in temperature-corrected electrical conductivity, consistent with past work indicating that spatially

localized inputs of alternate electron acceptors and other ions via nested groundwater flowpaths in calcareous rich fens can be seasonally fairly stable. The persistence of spatial patterns of sulfide and Fe(II) across seasons in a groundwater-fed wetland is biogeochemically significant in that it simplifies annualized estimates of causes and effects and lays the groundwork for more detailed studies of the controlling characteristics of the hotspots and lowspots of phytotoxic sulfide and Fe(II).

Introduction

Temperate zone biogeochemical field research often focuses on the warm-season as a consequence of academic-year schedules and cold-season logistical constraints such as snow-covered plots and frozen samples or equipment. Failing to adequately take into account seasonal variation from the warm-season to the cold-season provides an incomplete and sometimes misleading picture of year-round processes. Increasingly, it is becoming clear that the cold-season is not as much of a biological dormant season as might have been assumed (Campbell et al. 2005).

In some groundwater-fed wetlands, known as fens, the water table elevation is commonly quite stable across warm and cold seasons, although there are some notable exceptions (Duval and Waddington 2011). This is in strong contrast to uplands and some wetlands such as vernal pools in which the winter season is often dry as well as cold. In rich fens that have a stable water table elevation year-round the cold-season versus warm season porewater chemistry differences can be examined without the confounding influence of seasonal moisture regimes. In this study I

examined seasonal variation in sulfide and Fe(II), which are linked to phosphorus availability and can themselves be toxic.

My first hypothesis was that the reduced chemical constituents sulfide and Fe(II) would accumulate year-round in the porewater of a rich fen. An argument against this hypothesis is that cold temperatures could almost entirely inhibit biological sulfate reduction to sulfide and Fe(III) reduction to Fe(II), as well as abiotic iron reduction. However, there are many arguments in favor of winter sulfide and Fe(II) accumulation. First, the heat-retaining capacity of water in wetlands and the delivery of groundwater from moderate or deep depths should moderate seasonal temperature extremes. Second, there are sulfur-reducing and iron-reducing microbes that are known to maintain activity at low temperatures, as low as -1.8 ^{o} C (Knoblauch and Jorgensen 1999) for sulfur reducers. Third, temperature affects oxidizers as well as reducers (Rabenhorst and Castenson 2005, Pallud and Van Cappellen 2006, Weston et al. 2006, Leonov and Chicherina 2008, Tupikina et al. 2009, Sanz-Lazaro et al. 2011), and if rates of oxidation and reduction are affected similarly then the net accumulation of sulfide and Fe(II) may be only moderately affected. Finally, seasonal temperature effects on sulfide and Fe(II) accumulation may be obscured or counteracted by other seasonal shifts in biogeochemical redox controls such as the supply of alternate electron acceptors and high quality organic substrate.

My second and more novel hypothesis was that spatially heterogeneous patterns of sulfide and Fe(II) accumulation would exist in the warm-season and would be largely preserved in the cold-season. This of course is contingent on the prior hypothesis that sulfide and Fe(II) continue to accumulate in the cold season, and that there is therefore not simply a homogenous surface of zero sulfide or Fe(II) in the cold season. Beyond that, the other necessary preconditions are the presence of spatial heterogeneity in the warm season and consistency in the

drivers of that heterogeneity across seasons.

Spatial patterns of sulfide and Fe(II) accumulation are controlled in part by heterogeneity in the availability of alternate electron acceptors (e.g., sulfate for sulfate reducing bacteria) and electron donors to microbes. Heterogeneity in porewater concentrations of alternate electron acceptors like sulfate can in turn be caused by fine-scale variation in groundwater inputs of ions associated with substrate stratigraphy and basin morphometry (Boomer and Bedford 2008). The fine-scale variations in organic matter that influence spatial patterns of sulfide and Fe(II) (Stockdale et al. 2009) are caused in part by the distribution of plants that contribute carbon in such forms as root exudates and senesced fine and coarse roots. The spatial pattern of plants further influences the spatial pattern of sulfide and Fe(II) via radial oxygen loss (Van der Welle et al. 2007) that alters rates of microbial oxidation and reduction.

Seasonal consistency or inconsistency in the spatial patterns of the controls discussed above should result in corresponding seasonal consistency or inconsistency in sulfide and Fe(II) accumulation. Consistent groundwater flowpaths delivering sulfate and other alternate electron acceptors to the same portions of a fen year-round could therefore contribute to seasonal persistence in sulfide and Fe(II) accumulation, while seasonal groundwater flowpath variations and even head reversals (Bailey 1998) could help reset sulfide and Fe(II) each season. In parallel, a large excess of labile carbon produced by plants during the growing season to serve as an electron donor during the subsequent months when the plants are not actively photosynthesizing could sustain spatial reduction and oxidation hotspots across seasons, whereas more rapid depletion of labile carbon in the early months of the cold-season could limit subsequent microbial activity and erase hotspots of sulfide and Fe(II) accumulation. Finally, if plants in patchy locations are not entirely dormant in the cold season then root respiration or radial oxygen

loss could continue to influence sulfide and Fe(II) accumulation, even if not as strongly as in the warm season.

I reiterate here that I hypothesized that sulfide and Fe(II) would continue to accumulate in rich fen porewater during the cold season, and that the accumulation of sulfide and Fe(II) in the cold season would show the same spatially heterogeneous pattern as in the warm season as a consequence of consistent inputs of alternate electron acceptors via groundwater and consistent inputs of organic matter substrate from perennial plants in fixed locations.

Methods

Study Site

All sampling took place at Junius Pond fen, near the eastern shore of Lowery Pond in Seneca County within central New York State (Godwin et al. 2002, Boomer and Bedford 2008). Junius Pond fen is classified as containing a mixture of marl fen, rich graminoid fen, and rich shrub fen (Olivero 2001). Rich fens are a type of peat-forming wetland in which ground water inputs are a substantial component of the water budget. As the ground water input increases, wetlands shift from poor fens to rich fens and become quite distinct from bogs that are fed almost entirely by precipitation. The bedrock geology of the site dates to the Late Silurian, and includes pockets of anhydrite and salt in dolomite and shale of the Syracuse Formation and Akron Dolostone, including the Salina Group (Fisher et al. 1970, Isachsen et al. 2000). The surficial geology is kame moraine with calcareous cement, typically 10-30 m thick (Cadwell et

al. 1991). New York State is one of many regions in the United States (Dean and Johnson 1989) and beyond where some locations have underlying bedrock chemistry that is rich in sulfur.

Field

I intensively sampled porewater chemistry within a grid of 400 sampling locations evenly spaced 2-m apart in 20 rows and 20 columns for a total spatial extent of 38m x 38m. The grid of sampling locations made use of a transect with known hydrology and variation in sulfur chemistry (Boomer and Bedford 2008). At each sampling location I installed a porewater sipper with a sample screen extending from 8 to 12 cm below the soil surface, for an average sampling depth of 10 cm. I collected porewater from each sipper using a hand syringe equipped with an in-line 0.45 uM filter. Sample collection was repeated in fall (October 24 – November 3, 2008), winter (February 13 - 19, 2009), and summer (May 26 - June 1, 2009). Within each sampling period, I interrupted field collection to process redox sensitive sulfide and ferrous iron samples. In the field, I placed subsamples in two 23 mL borosilicate glass scintillation vials. In one vial I immediately mixed a 11.5 mL sample with 11.5 mL of previously-added sulfide anti-oxidant buffer (SAOB), filling the vial to capacity to minimize headspace, and set aside this sample aside for later sulfide analysis by ion selective electrode (ISE) in the lab as soon as possible. The SAOB was composed of NaOH, EDTA, and ascorbic acid to stabilize sulfide as S^{2-} to avoid oxidation to sulfate or volatile loss as H_2S. In a second vial I mixed 15 mL of sample with 5 mL of Ferrozine and HEPES reagents (Whitmire and Hamilton 2008) for ferrous iron analysis in the lab (Lovley and Phillips 1987). An additional sample was used to measure field pH, conductivity, and temperature with a portable multimeter and then discarded.

Lab and Data Analyses

In the lab, I measured porewater sulfide as S^{2-} with an ion selective electrode (ISE) using sodium sulfide standards calibrated by lead perchlorate and then speciated sulfide into H_2S and HS^- following (A.P.H.A. 2005). I measured porewater Fe(II) (ferrous iron) spectrophotometrically at an absorbance of 562 nm (Stookey 1970, Lovley and Phillips 1987).

Single-season spatial sulfide and Fe(II) data as well as change statistics across seasons were mapped using ArcGIS (version 9). Correlations of sulfide and Fe(II) across seasons were performed using R (version 2.14) software.

Results

Average porewater chemistry and temperature varied with season. Not surprisingly, porewater temperature decreased from summer to winter, with fall intermediate (Table 3-1). Winter porewater temperature sometimes fell to 0 ^{o} C but sippers froze solid in at most 3% of locations; probably less if a few sippers plugged with particulate rather than ice. Average porewater sulfide, Fe(II), and temperature-corrected conductivity were lowest in the winter, and pH was highest in the winter (Table 3-1). Average sulfide was highest in summer, Fe(II) was highest in fall, and pH and temperature-corrected conductivity were comparable in summer and fall (Table 3-1).

In the winter sampling period, sulfide and Fe(II) were still detectable in a majority of the

sampling locations (Figure 3-1). Sulfide and Fe(II) were spatially heterogeneous, with high sulfide locations usually distinct from high Fe(II) locations (Figure 3-1). Spatial patterns of sulfide and Fe(II) accumulation in winter were comparable to patterns in summer (Chapter 4). Most individual locations had lower sulfide and Fe(II) in winter than in summer (Figure 3-2), and the locations with the greatest seasonal change (Figure 3-2) were largely those with highest winter concentrations (Figure 3-1).

Summer sulfide and Fe(II) concentrations from summer (log10 transformed) were correlated with fall and winter concentrations (log10 transformed). Summer sulfide was correlated with winter sulfide (r^2=0.44) and fall sulfide (r^2=0.50) (Figure 3-3a). Summer Fe(II) was correlated with winter Fe(II) (r^2=0.34) and fall Fe(II) (r^2=0.60) (Figure 3-3b).

Table 3-1. Mean (+/- SD) porewater temperature, total sulfide, dihydrogen sulfide, ferrous iron, pH, and temperature corrected conductivity for each season (n=400 sample locations for each season).

Season	Temp. (^{o}C)	$HS^{-} + H_2S$ (uM)	H_2S (uM)	Fe(II) (uM)	pH	Cond. $_{TC}$ (uS/cm)
Summer	16.8 (2.1)	19.6 (38.4)	8.1 (16.1)	19.6 (26.9)	7.10 (0.22)	608 (102)
Fall	9.7 (2.3)	8.0 (13.6)	3.4 (6.0)	35.1 (60.3)	7.06 (0.19)	620 (109)
Winter	2.3 (1.2)	2.8 (6.4)	0.8 (1.9)	5.6 (13.3)	7.40 (0.16)	515 (107)

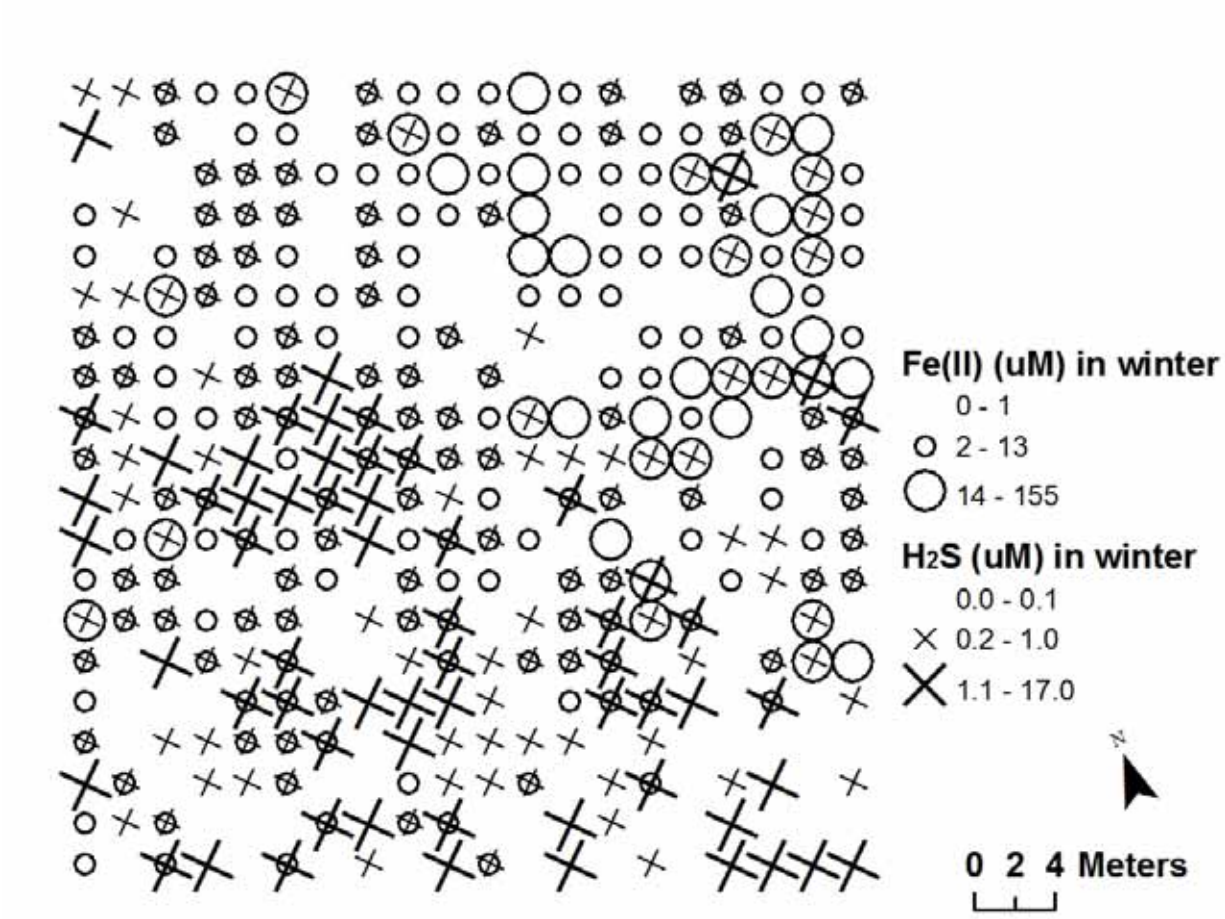


Figure 3-1. Winter sulfide and Fe(II) porewater chemistry (n=400 sample locations).

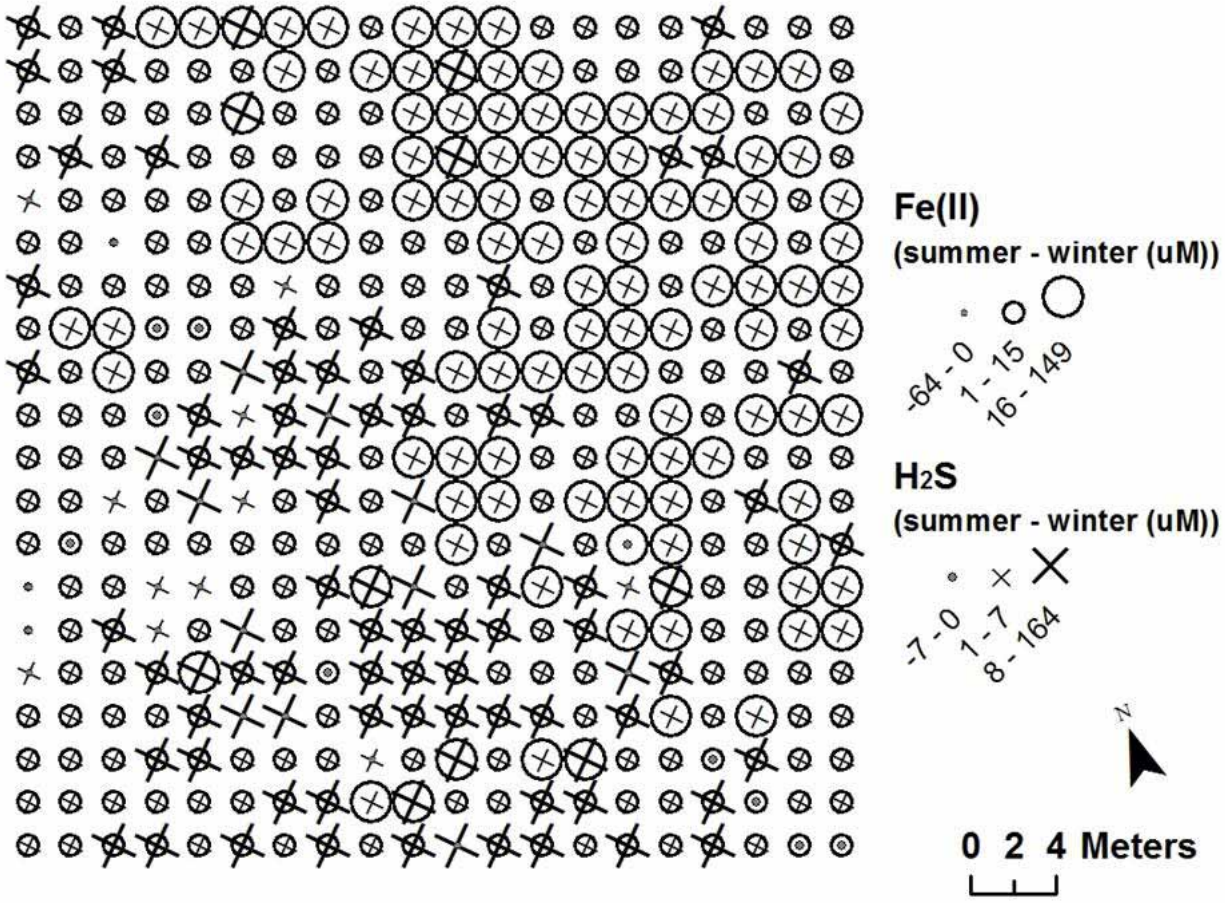
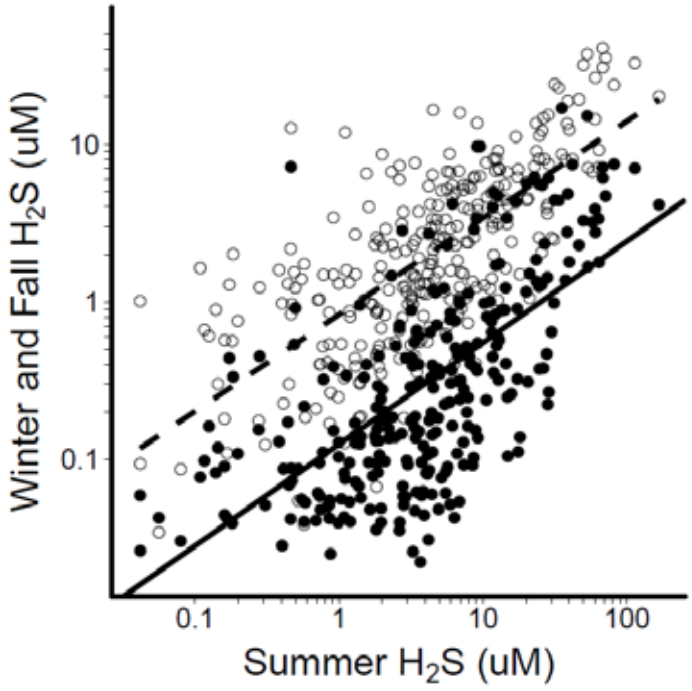
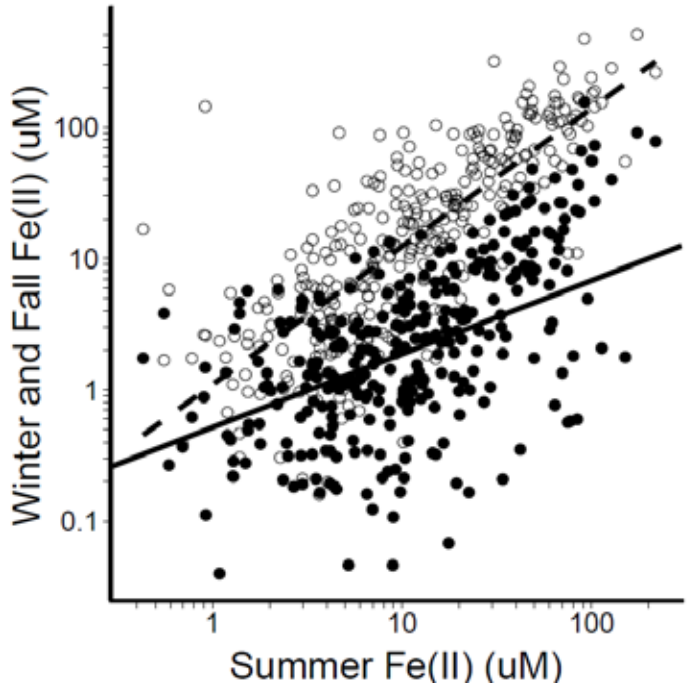


Figure 3-2. Seasonal change in sulfide and Fe(II) porewater chemistry (n=400 sample locations).



(a)



(b)

Figure 3-3. (a) Correlation of sulfide between seasons (n=400 sample locations for each season). (b) Correlation of Fe(II) between seasons (n=400 sample locations for each season). Closed circles with solid line are winter data and open circles with dashed line are fall data.

Discussion

SPATIAL PERSISTENCE OF SULFIDE AND FE(II) PATTERNS

As expected, the spatial pattern of porewater sulfide and Fe(II) accumulation persisted across seasons. The previously demonstrated delivery of sulfate and other alternate electron acceptors via nested groundwater flowpaths (Boomer and Bedford 2008) through fixed-location channels of substrate with high hydraulic conductivity is likely to have been a key contributor to this seasonally persistent pattern of redox chemistry. The stabilizing influence of groundwater is supported by the seasonally persistent spatial pattern and modest absolute seasonal change in temperature corrected electrical conductivity. Another likely contribution to the persistent spatial pattern of sulfide and Fe(II) is the presence of immobile perennial plants that supply sulfur and iron reducers with organic substrate in fixed locations in the form of root exudates or sloughed-off particulates from roots. Unfortunately, I can not evaluate the potential importance of spatial patterns of organic matter on the observed pattern of sulfide and Fe(II) accumulation since I did not measure organic matter quantity or quality. Having established that the spatial pattern of sulfide and Fe(II) can persist across seasons, additional research to clarify the mechanisms of that pattern would be a logical next step.

WINTER SULFIDE AND FE(II) WAS NOT A HOMOGENOUS SURFACE OF ZEROS

A spatial pattern of sulfide and Fe(II) would not have persisted in winter if low temperatures or other factors had entirely suppressed sulfur and iron reduction. Despite winter air

temperatures that were less than 0 ° C, winter water temperature was maintained at an average of 2.3 ° C by groundwater inputs that are more likely than surface water to mirror long-term average temperatures. Despite near-zero temperatures, sulfide continued to accumulate in winter, possibly as a consequence of several of the speculated explanations below. The activity of cold-tolerant species of sulfate and sulfur reducing bacteria may have increased in winter. A more parsimonious explanation would be that microbial sulfate reduction did decrease dramatically in the winter but decreased only moderately more than microbial sulfide oxidation. Another possibility is that winter decreases in microbial sulfate reduction were partially compensated by decreases in abiotic sulfide oxidation due to minimal or no radial oxygen loss by dormant plants in winter. There is likely an analogous set of possible explanations for the persistence of Fe(II) in winter. Further research would clarify whether these or other mechanisms were responsible for the existence of a winter spatial pattern of sulfide and Fe(II) accumulation rather than a homogenous surface of zeros.

AN ADDITIONAL NON-SPATIAL SEASONAL SHIFT IN CHEMISTRY

Spatial patterns of sulfide and Fe(II) across seasons were the focus of this study, but one seasonal change that was not spatially explicit deserves attention here. The peak accumulation of Fe(II) in fall rather than summer highlights the fact that temperature is just one of many controls over the accumulation of reduced chemical species. Plant-mediated radial oxygen loss influences rates of iron reduction (Neubauer et al. 2005). Seasonal changes in carbon within the rhizosphere are also likely a factor. In the fall the most labile carbon source may have shifted from root exudates to senescing fine roots, with iron reducers showing an increased response or iron

oxidizers showing a decreased response compared to sulfate oxidizers and sulfide oxidizers. Any slight seasonal increase in Fe(II) could also be amplified by any decrease in sulfide and subsequent decrease in the quantity of iron precipitated as iron sulfide.

FUTURE RESEARCH

Having demonstrated that spatial patterns of porewater sulfide and Fe(II) persist across seasons, additional winter research could target mechanistic environmental controls of sulfur and iron reduction and oxidation in biogeochemical hotspots and lowspots of sulfide and Fe(II) accumulation. In particular, the influence of carbon quantity and quality on accumulation of sulfide and Fe(II) in winter would be a high priority.

This research exploring the spatial pattern of sulfide in winter could also be extended to address the potential influence of sulfide on methanogenesis on an annual basis. Sulfate reduction to sulfide may inhibit methanogenesis under certain restricted circumstances (Gauci et al. 2004), but organic substrate is often more important than sulfate reduction in influencing rates of methanogenesis (Yavitt and Lang 1990). An integrated and spatially intensive assessment of carbon quality, rates of sulfate reduction, and rates of methanogenesis across seasons could not only clarify the extent of any winter sulfide impact on annual methane fluxes but also pinpoint what fraction of the area of a groundwater-fed wetland's area is responsible for most of the methane emissions.

SUMMARY AND SIGNIFICANCE

Spatial patterns of porewater sulfide and Fe(II) accumulation were persistent across seasons in a groundwater-fed rich fen wetland. This is ecologically significant since it means that fen plants that must tolerate sulfide or Fe(II) in the warm season do not have a seasonal refugia from these phytotoxic reduced species in the cool season either. Biogeochemically, the spatial persistence of sulfide and Fe(II) across seasons makes it more feasible to scale short-term biogeochemical measurements to an annual basis.

REFERENCES

A.P.H.A. 2005. 4500 - S^{2-} (SULFIDE). Page 4.170 to 174.183 *in* A. D. Eaton, L. S. Clesceri, E. W. Rice, and A. E. Greenberg, editors. Standard methods for the examination of water & wastewater. American Public Health Association, Washington, DC.

Bailey, K. M. 1998. Hydrology and water chemistry patterns driven by short-term geomorphic processes in a freshwater peatland. Cornell University, Ithaca, NY.

Boomer, K. M. B. and B. L. Bedford. 2008. Influence of nested groundwater systems on reduction-oxidation and alkalinity gradients with implications for plant nutrient availability in four New York fens. Journal of Hydrology 351:107-125.

Cadwell, D. H., G. G. Connally, P. J. Fleisher, E. H. Muller, R. A. Young, R. J. Dineen, J. L. Rich, M. L. Fuller, L. Sirkin, G. C. Wiles, D. L. Pair, D. A. Franzi, J. T. Gurrieri, G. M. Haselton, G. C. Kelley, R. G. LaFleur, and J. S. Street. 1991. Surficial Geologic Map of New York consisting of five sheets: Finger Lakes, Hudson-Mohawk, Niagara, Lower Hudson and Adirondack. New York State Geological Survey Map and Chart Series. New York State Museum / New York State Geological Survey, Albany.

Campbell, J. L., M. J. Mitchell, P. M. Groffman, L. M. Christenson, and J. P. Hardy. 2005. Winter in northeastern North America: a critical period for ecological processes. Frontiers in Ecology and the Environment 3:314-322.

Dean, W. E. and K. S. Johnson. 1989. Anhydrite Deposits of the United States and Characteristics of Anhydrite Important for Storage of Radioactive Wastes: An inventory of the geologic, physical, hydrologic, and chemical characteristics of anhydrite deposits in the United States and evaluation of anhydrite as a possible medium for the storage of radioactive wastes. USGS Bulletin 1794. United States Government Printing Office, Denver, CO.

Duval, T. P. and J. M. Waddington. 2011. Extreme variability of water table dynamics in temperate calcareous fens: Implications for biodiversity. Hydrological Processes 25:3790-3802.

Fisher, D. W., Y. W. Isachsen, and L. V. Rickard. 1970. Geologic Map of New York State, 1970, consisting of 5 sheets: Niagara, Finger Lakes, Hudson-Mohawk, Adirondack, and Lower Hudson. New York State Geological Survey Map and Chart Series. New York State Museum / New York State Geological Survey, Albany.

Gauci, V., E. Matthews, N. Dise, B. Walter, D. Koch, G. Granberg, and M. Vile. 2004. Sulfur pollution suppression of the wetland methane source in the 20th and 21st centuries. Proceedings of the National Academy of Sciences of the United States of America 101:12583-12587.

Godwin, K. S., J. P. Shallenberger, D. J. Leopold, and B. L. Bedford. 2002. Linking landscape properties to local hydrogeologic gradients and plant species occurrence in minerotrophic fens of New York State, USA: A Hydrogeologic Setting (HGS) framework. Wetlands 22:722-737.

Isachsen, Y. W., E. Landing, J. M. Lauber, L. V. Rickard, and W. B. Rogers. 2000. Geology of New York: a simplified account. Second Edition edition. New York State Museum / Geological Survey, State Education Dept., University of the State of New York, Albany, NY.

Knoblauch, C. and B. B. Jorgensen. 1999. Effect of temperature on sulphate reduction, growth rate and growth yield in five psychrophilic sulphate-reducing bacteria from Arctic sediments. Environmental Microbiology 1:457-467.

Leonov, A. and O. Chicherina. 2008. Sulfate reduction in natural water bodies. 1. The effect of environmental factors and the measured rates of the process. Water Resources 35:417-434.

Lovley, D. R. and E. J. P. Phillips. 1987. Rapid Assay For Microbially Reducible Ferric Iron In Aquatic Sediments. Applied and Environmental Microbiology 53:1536-1540.

Neubauer, S. C., K. Givler, S. K. Valentine, and J. P. Megonigal. 2005. Seasonal patterns and plant-mediated controls of subsurface wetland biogeochemistry. Ecology 86:3334-3344.

Olivero, A. M. 2001. Classification and Mapping of New York's Calcareous Fen Communities. New York Natural Heritage Program, Albany, NY.

Pallud, C. and P. Van Cappellen. 2006. Kinetics of microbial sulfate reduction in estuarine sediments. Geochimica Et Cosmochimica Acta 70:1148-1162.

Rabenhorst, M. C. and K. L. Castenson. 2005. Temperature effects on iron reduction in a hydric soil. Soil Science 170:734-742.

Sanz-Lazaro, C., T. Valdemarsen, A. Marin, and M. Holmer. 2011. Effect of temperature on

biogeochemistry of marine organic-enriched systems: implications in a global warming scenario. Ecological Applications 21:2664-2677.

Stockdale, A., W. Davison, and H. Zhang. 2009. Micro-scale biogeochemical heterogeneity in sediments: A review of available technology and observed evidence. Earth-Science Reviews 92:81-97.

Stookey, L. L. 1970. Ferrozine - A New Spectrophotometric Reagent For Iron. Analytical Chemistry 42:779-781.

Tupikina, O. V., V. A. Rassulov, and T. F. Kondrat'eva. 2009. Patterns of pyrite oxidation by different microorganisms. Microbiology 78:165.

Van der Welle, M. E. W., K. Niggebrugge, L. P. M. Lamers, and J. G. M. Roelofs. 2007. Differential responses of the freshwater wetland species *Juncus effusus* L. and *Caltha palustris* L. to iron supply in sulfidic environments. Environmental Pollution 147:222-230.

Weston, N., W. Porubsky, V. Samarkin, M. Erickson, S. Macavoy, and S. Joye. 2006. Porewater Stoichiometry of Terminal Metabolic Products, Sulfate, and Dissolved Organic Carbon and Nitrogen in Estuarine Intertidal Creek-bank Sediments. Biogeochemistry 77:375.

Whitmire, S. L. and S. K. Hamilton. 2008. Rates of anaerobic microbial metabolism in wetlands of divergent hydrology on a glacial landscape. Wetlands 28:703-714.

Yavitt, J. B. and G. E. Lang. 1990. Methane production in contrasting wetland sites - Response to organic-chemical components of peat and to sulfate reduction. Geomicrobiology Journal 8:27-46.

CHAPTER 4

ENVIRONMENTAL GRADIENTS OF SULFUR, IRON, PHOSPHORUS AND CALCIUM AND FINE SCALE PLANT HETEROGENEITY WITHIN A GROUNDWATER-FED WETLAND

Abstract

Plant diversity in groundwater-fed wetlands is typically extraordinarily high, yet the biogeochemical controls of this diversity are still incompletely understood. I hypothesized that fine-scale variation in sulfide would influence plant community composition via direct phytotoxicity and indirect mediation of phosphorus release from iron, coupled with gradients in other chemical constituents such as calcium. I measured porewater chemistry and associated plant species composition at 400 locations within a calcareous rich fen. Groundwater-derived calcium and sulfate and redox-sensitive sulfide and ferrous iron showed high heterogeneity. Phosphorus availability was limited and not readily traceable to toxic sulfide, while nitrogen (TDN) was a more abundant nutrient than expected. Using AICc criteria to select between competing models of toxin, nutrient, and mixed-chemistry influences on vegetation, I found that hydrogen sulfide reduced total plant cover, cover of the three most frequently occurring species, dicot species density, plant height, and litter accumulation. Sulfide coupled with calcium and phosphorus to influence plant species composition. Sulfide was typically more likely to reduce the cover of common species than uncommon species. Presence and cover of uncommon species was often unexpectedly explained best by Fe(II) and sulfate, but for all species and plant

response variables the models that included environmental predictor variables fit the data better than the "null" model of mean plant response that lacked environmental variables. An integrated geochemical assessment of coupled groundwater chemistry, redox-sensitive chemistry, and nutrient influences on plants helped explain high heterogeneity in plant species composition and diversity.

Introduction

Sulfide should have a strong influence on plant species composition, given the direct toxicity of sulfide (Bagarinao 1992) and indirect sulfide-mobilization of iron-bound phosphorus in anoxic environments (Caraco et al. 1989). Empirically, the importance of sulfide to wetland plants is well demonstrated in coastal wetlands (Chambers 1997) and in inland wetlands impacted by agriculture in the Netherlands (Lamers et al. 1998), but has received surprisingly little attention in most of the world's inland wetlands. Most likely, the dichotomy between sulfate-rich coastal waters and comparatively more sulfate-poor inland waters has obscured the wide range of sulfate and sulfide that plants encounter within inland wetlands. Variations in geological composition (Dean and Johnson 1989), atmospheric deposition (Weathers et al. 2006), and agricultural runoff (Lamers et al. 1998) create a highly heterogeneous landscape of inland wetland sulfur supply. Variability in rates of sulfate reduction (Wieder et al. 1990) and sulfide oxidation (Almendinger and Leete 1998) due to variables such as carbon quality (Sutton-Grier et al. 2011) and temperature (Sanz-Lazaro et al. 2011) should create even greater heterogeneity in the toxic sulfide form of sulfur. Despite expected sulfide heterogeneity and the known toxic and nutrient-releasing roles of sulfide, little is known about the influence of sulfide heterogeneity on plant species composition and diversity.

Sulfide's dual influences on plants as toxin and mediator of internal phosphorus release should be a general phenomenon in inland wetlands worldwide, even though most documentation to date comes from wetlands of the Netherlands (Lamers et al. 1998, Geurts et al. 2009). A wide-ranging and ubiquitous influence of sulfide heterogeneity on plant species composition would be a logical extension of the continuous expansion of the number of recognized environmental dimensions in wetland ecosystems (Bridgham et al. 1996). Sulfide has long been recognized as a plant toxin (McCallan et al. 1936). Like cyanide, sulfide interferes with aerobic respiration by binding the terminal cytochrome c oxidase enzyme in the mitochondria (Bagarinao 1992). The indirect influence of sulfide on phosphorus availability to wetland plants can be traced to linked hydrologic and redox gradients (Boomer and Bedford 2008b). Any change in phosphorus availability is critical since plant growth in many wetlands is limited by phosphorus or co-limited by phosphorus and nitrogen (Bedford et al. 1999). Individually, differentiation among plant species in capacity to avoid or tolerate sulfide toxicity in either seedling or adult life stages or to utilize phosphorus indirectly mobilized by sulfide should alter competitive outcomes. Collectively, independent gradients of sulfide toxicity and phosphorus release should produce a heterogeneous patchwork of microsites supporting a corresponding heterogeneous and diverse plant community.

The linkage between sulfide and phosphorus is iron. When sulfide reduces Fe(III) to Fe(II) it mobilizes phosphate that was formerly bound to Fe(III) (Figure 4-1a). However, iron also has a direct impact on plants via iron toxicity in the Fe(II) form (Snowden and Wheeler 1993), and can also influence phosphorus uptake via iron plaques (Xu et al. 2009). If sulfide does not completely precipitate Fe(II) out of solution as iron monosulfide (FeS) or pyrite (FeS_2), then plants have to ameliorate sulfide and Fe(II) toxicity simultaneously (van der Welle et al. 2006).

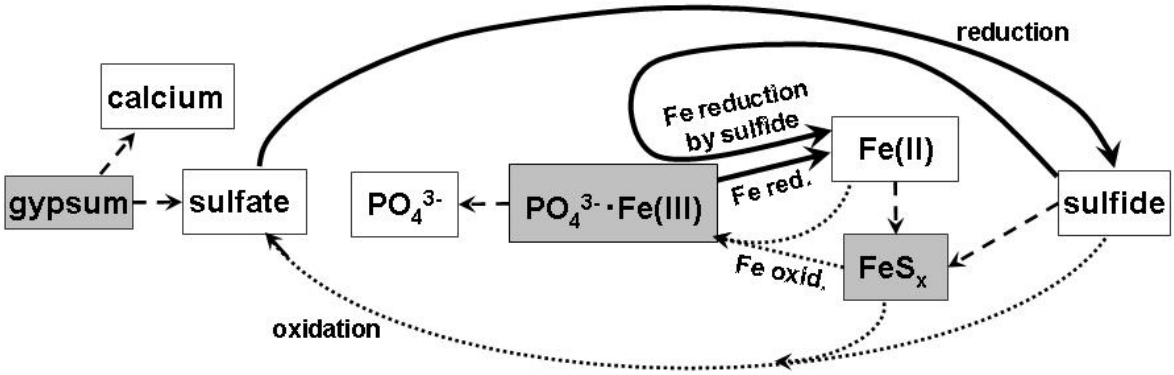
Unlike sulfide, Fe(II) primarily enters plants in aqueous phase and is involved in free radical formation (Becana et al. 1998, Gross et al. 2003, Dufey et al. 2009). If sulfide and Fe(II) tolerance traits are inherited independently and H_2S and Fe(II) environmental exposure are not collinear, then these two reduced species should contribute to the diversity of plants found in some wetlands. As with sulfide versus phosphorus, there has not to my knowledge been any previous attempt to map out spatial patterns of sulfide and Fe(II) and relate them to spatial variability in plant species composition.

Many wetlands receive both sulfate and calcium in groundwater. Calcareous rich fens are, among other locations, present in the upper Midwest and Northeastern regions of the United States. Calcium is itself an influence on plant performance directly (White and Broadley 2003) and via links to iron and phosphorus (Zohlen and Tyler 2000). Fortunately, it is possible to address the potentially confounding influences of calcium and sulfide on plant species composition by making use of geological variations in calcium sulfate and calcium carbonate substrate that exist between and even within some sites.

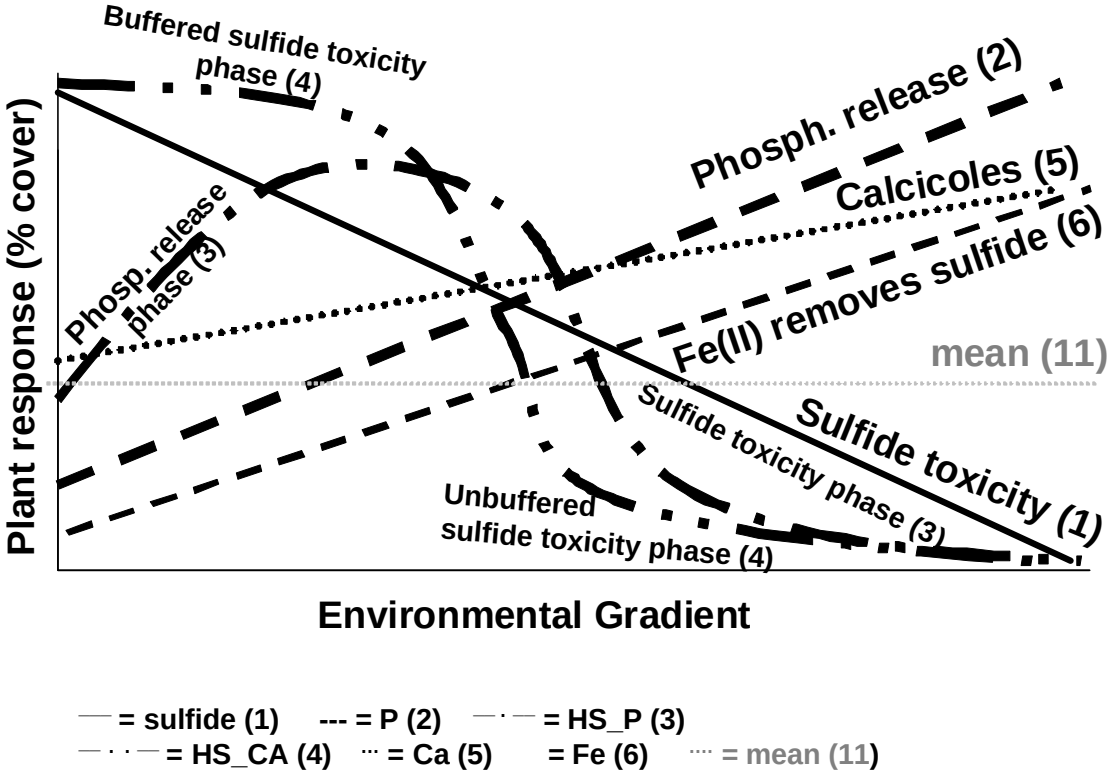
I formulated a suite of hypotheses representing some of the diversity of possible relationships between porewater chemistry and plant community composition (Figure 4-1b and Table 4-1). My focus in this research is the direct and indirect relationship of sulfide to wetland plant community composition, but I addressed related biogeochemical elements as well. The favored hypothesis was that direct sulfide toxicity to plants inhibits establishment of sensitive species at the seedling stage and/or allows competitive dominance of insensitive species during the adult stage, trumping all other biogeochemical influences (Hypothesis 1). Alternatively, indirect sulfide mobilization of phosphorus and subsequent release of plants from phosphorus limitation (Hypothesis 2) may overwhelm any direct toxicity effect. If direct sulfide toxicity and

indirect phosphorus release both occur, then the net effect on plants may depend on the relative magnitude of these two effects (Hypothesis 3). These first three hypotheses were the most favored *a priori*, based on (Lamers et al. 1998). However, given the strong calcium signature in many groundwater fed ecosystems and the literature addressing calcicoles versus calcifuges discussed above, I considered the possibility that calcium either in tandem with sulfide (Hypothesis 4) or in isolation (Hypothesis 5) was the dominant influence on vegetation, perhaps in keeping with conventional wisdom. I deemed the remaining hypotheses addressing direct Fe(II) toxicity (Hypothesis 6), alternate indirect indicators of phosphorus status (Hypothesis 7), combined atmospheric deposition of sulfur and nitrogen (Hypothesis 8), and nitrogen-phosphorus nutrient co-limitation (Hypothesis 9) to be plausible but considerably less likely to predominate in a groundwater-fed wetland ecosystem. For the sake of completeness and to balance my set of hypotheses, I included the hypothesis that all environmental predictor variables except the two favored sulfide and phosphorus variables provided the best explanation of plant species composition (Hypothesis 10). Lastly, I considered the hypothesis that none of the measured environmental variables helped explain a plant response variable better than the mean of that response (Hypothesis 11), crudely interpretable as a rough approximation of the null hypothesis in a frequentist null-hypothesis test framework. By evaluating the relative weight of these hypotheses I determined if and to what extent sulfide plays a role in plant species composition. I evaluated these hypotheses in a calcareous rich fen, but with appropriate modifications these hypotheses should be relevant in other wetland ecosystems.

Methods



(a)



(b)

Figure 4-1. (a) Conceptual model of linked biogeochemical cycling of sulfur, phosphorus, and iron in wetlands. Porewater constituents are illustrated with open boxes, solid phase chemistry is illustrated with shaded boxes, solid arrows illustrate reduction reactions, dotted lines illustrate oxidation reactions, and dashed arrows illustrate precipitation of chemicals. (b) Conceptual diagram of expected plant response to biogeochemically-controlled water chemistry gradients. Hypotheses 1-6 and 11 are stylized representations of those in Table 4-1.

Table 4-1. Competing ecological hypotheses linking plant response to environmental variable(s). The general form of each equation is $\hat{y} = a + b*X + \varepsilon_{i,}$, where $\hat{y}$ is the predicted value of a plant response variable, a is the intercept, X is the first environmental predictor variable, b is the parameter estimate of variable X, and ε_i is the normally distributed residual error. Each of the 11 models are applied separately to each of the following plant response variables ($\hat{y}$): percent cover of each plant species, percent cover of each plant functional group, species density, plant height, percent bare ground, and percent litter. The first three models highlighted in bold were the favored *a priori* models but the other models were included to represent other competing models from the literature and to balance the model set.

Hypothesis ID	Hypothesis Description	Model/Equation
1) **HS**	Sulfide toxicity	$\hat{y} = a + b*HS + \varepsilon_{i,}$ where HS= single toxin sulfide
2) **P**	Phosphorus released to plants	$\hat{y} = a + b*P + \varepsilon_{i,}$ where P=single nutrient phosphorus
3) **HS_P**	Linked sulfide toxicity and phosphorus nutrient release	$\hat{y} = a + b*HS + c*P + \varepsilon_i$ where HS=sulfide and P=phosphorus
4) HS_CA	Linked sulfide toxicity and groundwater discharge proxy	$\hat{y} = a + b*HS + c*CA + \varepsilon_i$ where HS=sulfide and CA=calcium
5) CA	Groundwater discharge proxy or calcicole	$\hat{y} = a + b*Ca + \varepsilon_{i,}$ where CA=calcium
6) FE2	Iron toxicity or alternate index of phosphorus release	$\hat{y} = a + b*FE2 + \varepsilon_{i,}$ where FE2=single toxin Fe(II)
7) FE2_SO4	Phosphorus release and desorption	$\hat{y} = a + b*FE2 + c*SO4 + \varepsilon_i$ where FE2=Fe(II) and SO4=SO_4^{2-}
8) SO4_N	Atmospheric deposition	$\hat{y} = a + b*SO4 + c*N + \varepsilon_i$ where SO4=SO_4^{2-} and N=TDN
9) N_P	Nitrogen and phosphorus both available	$\hat{y} = a + b*N + c*P + \varepsilon_i$ where N=TDN and P=phosphorus
10) CA_FE2_SO4_N	Anything but sulfide toxicity or phosphorus release	$\hat{y} = a + b*CA + c*FE2 + d*SO4 + e*N + \varepsilon_i$
11) mean	Measured environmental variables not related to plant response variable.	$\hat{y} = \bar{y} + \varepsilon_i$ where $\bar{y}$ =mean of plant response

STUDY SITE

A fen is considered here to be a type of peat-forming wetland with an abundance of brown mosses in which groundwater inputs are a substantial component of the water budget. As the ground water input increases, wetlands shift from poor fens to rich fens with an abundance of brown mosses and become quite distinct from bogs that are fed almost entirely by precipitation and are characterized by an abundance of *Sphagnum* mosses. The dominant influence of ground water in rich fens makes them an ideal study system for addressing the impact of geochemistry on plant community composition. Specifically, I consider rich fens to be model systems for addressing the influence of sulfide on plant community composition since sulfate is decoupled from sodium and chloride yet there are still sufficiently large gradients of sulfate and sulfide from geological origins to readily detect changes with respect to plant communities.

The site of this study was Junius Pond fen, near the eastern shore of Lowery Pond in Seneca County within central New York State (Godwin et al. 2002, Boomer and Bedford 2008b). Junius Pond fen is classified as containing a mixture of marl fen, rich graminoid fen, and rich shrub fen (Olivero 2001). The bedrock geology of the site dates to the Late Silurian, and includes pockets of anhydrite and salt in dolomite and shale of the Syracuse Formation and Akron Dolostone, including the Salina Group (Fisher et al. 1970, Isachsen et al. 2000). The surficial geology is kame moraine with calcareous cement, typically 10-30 m thick (Cadwell et al. 1991). New York State is one of many regions in the United States (Dean and Johnson 1989) and beyond where some locations have underlying bedrock chemistry which is rich in sulfur. I focus here on a groundwater fed rich fen but suggest that sulfide deserves additional attention in other ecosystems as well.

FIELD SAMPLING

In early summer of 2009 I intensively sampled water chemistry and plant community composition within a grid of 400 sampling locations evenly spaced 2-m apart in 20 rows and 20 columns for a total spatial extent of 38m x 38m (Figure 4-2). The grid of sampling locations made use of a transect with known hydrology and variation in sulfur chemistry (Boomer and Bedford 2008b). At each sampling location I measured plant community composition and porewater chemistry. I recorded percent cover of each vascular and bryophyte species, bare, litter, and open water (if applicable), the height of the overall tallest species, and the height of the tallest *Eleocharis rostellata* individual in a small 0.1 m x 0.1 m quadrat from 6–14 June 2009. In the center of each vegetation quadrat I installed a porewater sipper with a sample screen extending from 8 to 12 cm below the soil surface, for an average sampling depth of 10 cm. For my purposes I sought to measure sulfide at a spatial scale matching a reasonable fraction of a plant's rhizosphere, for which I assumed a bulk interstitial porewater sipper would be appropriate.

I collected porewater from each sipper using a hand syringe equipped with an in-line 0.45 uM filter between 26 May and 1 June 2009, interrupting field collection to process redox sensitive sulfide and ferrous iron samples. In the field, I placed subsamples in three 23 mL borosilicate glass scintillation vials. In one vial I immediately mixed a 11.5 mL sample with 11.5 mL of previously-added sulfide anti-oxidant buffer (SAOB), filling the vial to capacity to minimize headspace, and set aside this sample aside for later sulfide analysis by ion selective electrode (ISE) in the lab as soon as possible. The SAOB was composed of NaOH, EDTA, and ascorbic acid to stabilize sulfide as S^{2-} to avoid oxidation to sulfate or volatile loss as H_2S. In a

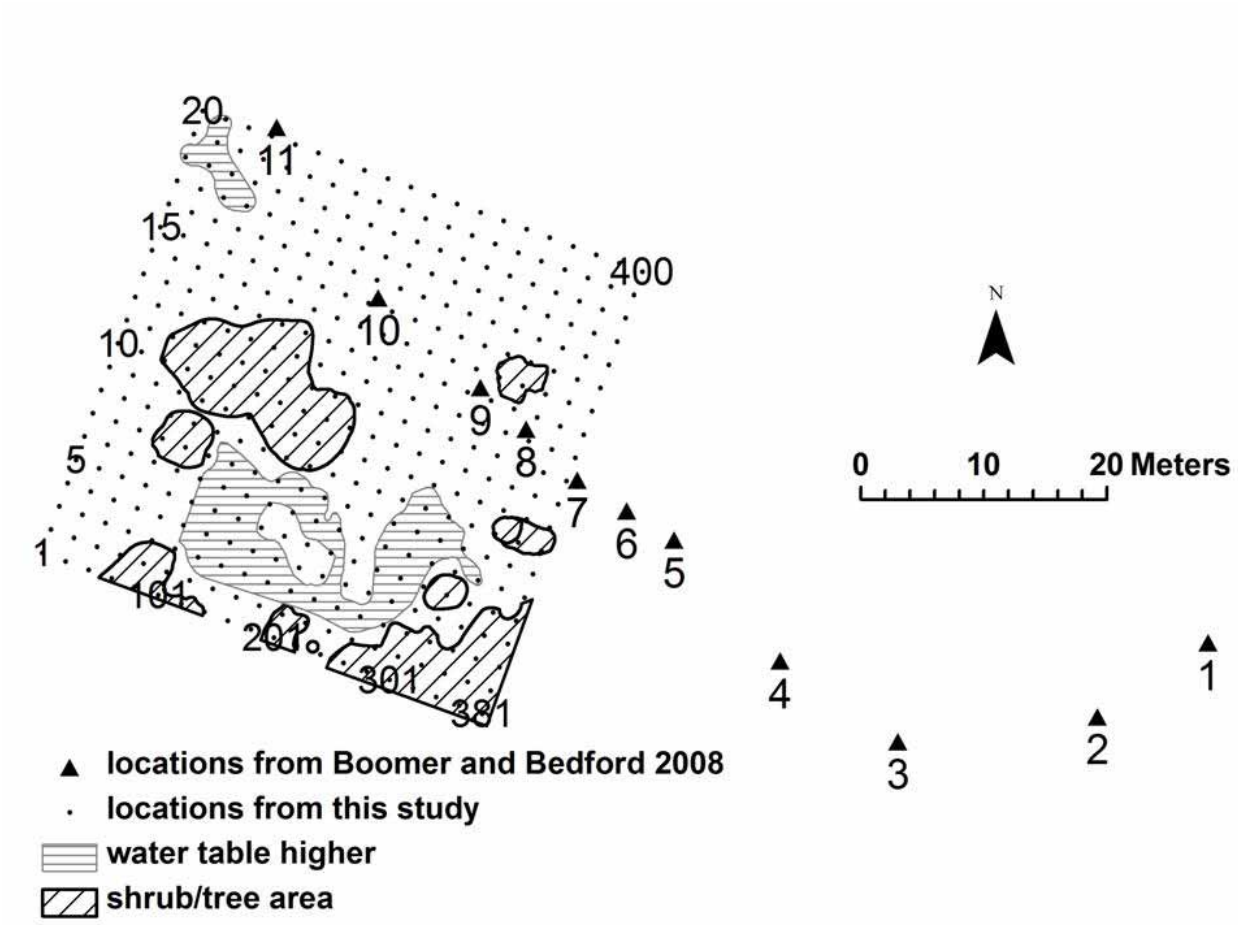


Figure 4-2. Site map of Junius Pond fen, NY showing the 400 sampling locations in this study (selected locations on margins of grid labeled), 11 locations from Boomer and Bedford (2008), areas with denser shrubs/trees, and areas with higher water table.

second vial I mixed 15 mL of sample with 5 mL of Ferrozine and HEPES reagents (Whitmire and Hamilton 2008) for ferrous iron analysis in the lab (Lovley and Phillips 1987). In a third vial I collected sample for later lab analysis of calcium, sulfate, and Total Dissolved Nitrogen (TDN). An additional sample was used to measure field pH, conductivity, and temperature with a portable multimeter and then discarded. Finally, I deployed a pair of 2.5 cm by 5 cm anion resin strips (GE Water & Process Technologies, product number AR204SZRA) at each sampling location on 10–11 May 2009 at a depth of 1-3 cm and retrieved the strips 8–12 June, 2009 for an index of phosphorus availability.

LAB ANALYSES

In the lab, I measured porewater sulfide as S^{2-} with an ion selective electrode (ISE) using sodium sulfide standards calibrated by lead perchlorate and then speciated sulfide into H_2S and HS^- following (A.P.H.A. 2005). I measured porewater Fe(II) (ferrous iron) spectrophotometrically at an absorbance of 562 nm (Stookey 1970, Lovley and Phillips 1987). Aqueous porewater samples were submitted to the Analytical Lab of the Cary Institute of Ecosystem Studies for inductively coupled plasma atomic-emission spectrometer (ICP-AES) analysis of calcium, ion chromatographic (IC) analysis of sulfate, and continuous-flow analysis (CFA) of Total Dissolved Nitrogen (TDN) (Weathers et al. 2001). I extracted resin strips with 0.5 M HCl (Crowley and Bedford 2011), then added Murphy-Riley reagents (sulfuric acid, ammonium molybdate, antimony potassium tartrate) and analyzed samples spectrophotometrically for phosphate at 880 nm (Murphy and Riley 1962).

STATISTICAL ANALYSES

The competing ecological models in Table 4-1 represent as closely as possible my multiple working hypotheses. The idea of multiple working hypotheses has been around for more than a hundred years (Chamberlain 1890, Anderson 2008). It was largely displaced by the frequentist null-hypothesis test framework but has been popularized again in an information theoretic approach (Chamberlain 1890, Anderson 2008) in an era where computing power makes it practical to implement. In the information theoretic framework there is no null hypothesis, but the model containing no environmental predictor variables (Hypothesis 11 in Table 4-1) is the closest to a null hypothesis. In order to evaluate the relative strength of evidence for competing ecological hypotheses in Table 4-1 I used R (version 2.14) software to calculate the log likelihood and complexity of each model using the corrected Akaike information criterion (AICc). Models with the lowest AICc values were taken to be the best fit given the data available. In addition to cover and species richness, I used the AIC framework to look at competing models explaining plant height (of the tallest species and also the most frequent species, *E.rostellata*) and percent bare ground as a function of environmental variables.

To address the possibility of fine-scale autocorrelation structure I examined semivariograms of model residual errors. Variation at even the shortest 2-m distance (the nugget) was 80% or more of the sill for the three plant functional groups, indicating that spatial autocorrelation of residuals was modest. Since spatial autocorrelation was modest I did not quantitatively incorporate spatial autocorrelation structure into models. Instead I addressed the more severe issue of non-normal distributions by incorporating a binomial process in the models, as discussed below.

Unlike plant functional groups, individual plant species percent cover values had zero-inflated distributions that could not be normalized, so I used a two-part modeling approach in which the first component of the model evaluates presence versus absence in a binomial distribution of the generalized linear model (GLM), while the second component of the model looks at square-root transformed cover values at only those locations where the species is present. I summed the maximum likelihoods of the two parts of the model and conservatively used the lower sample size of the cover analysis in the second part of the model to calculate AICc information criteria values. Subsequently for each species I identified the model with the least information loss (lowest AICc value) and subtracted that from the AICc value of the other ten models to calculate the ΔAICc of each model. The model set was balanced with regard to the number of times that each of the six environmental values (sulfide, phosphorus, calcium, Fe(II), sulfate, and nitrogen) occurred in a model, permitting calculation of model weights from ΔAICc (Anderson 2008) values as follows: model weight =exp(-ΔAICc/2)/ sum(exp(-ΔAICc/2))). The sum of the model weights totals one for each species, and an individual model with a higher model weight has a better fit to the model, given the data. Finally, I used model weights to calculate average parameter estimates, given the entire model set (Anderson 2008). Note that model weights alone do not give the magnitude or direction of influence of component environmental variables. This latter piece of information can only be obtained from the weighted parameter estimates.

In order to visualize variation in plant community composition and relate it to environmental gradients I ordinated plots in plant species space using nonmetric multidimensional scaling (NMS), a multivariate analysis that is still robust when there are many zero values, as is common with plant community data (McCune and Grace 2002). I

implemented NMS with function metaMDS of the R package vegan (Oksanen et al. 2011), using Bray-Curtis/Sorensen distances, a starting configuration derived from metric scaling, 200 data runs, and three dimensions. I removed species occurring in less than 5% of plots (McCune and Grace 2002), and remaining species cover values were square-root transformed. Axes scores were centered, rotated to align with PC axes, and halfchange scaled. I sought a convergent solution in 200 runs, and failing that I used the solution with the lowest stress as the final solution. I added scaled species correlations with the first two NMS axes and calculated the linear correlations of those axes with the following environmental variables: total sulfide (H_2S plus HS^-), resin phosphorus, calcium, ferrous iron, sulfate, total dissolved nitrogen, temperature corrected conductivity, and pH. On top of the ordination I overlaid nonlinear contours of sulfide and calcium using nonlinear fitting of thin plate splines in a General Additive Model (GAM) framework, implemented with function ordisurf of the R package vegan (Oksanen et al. 2011).

Results

POREWATER CHEMISTRY AND PATTERNS

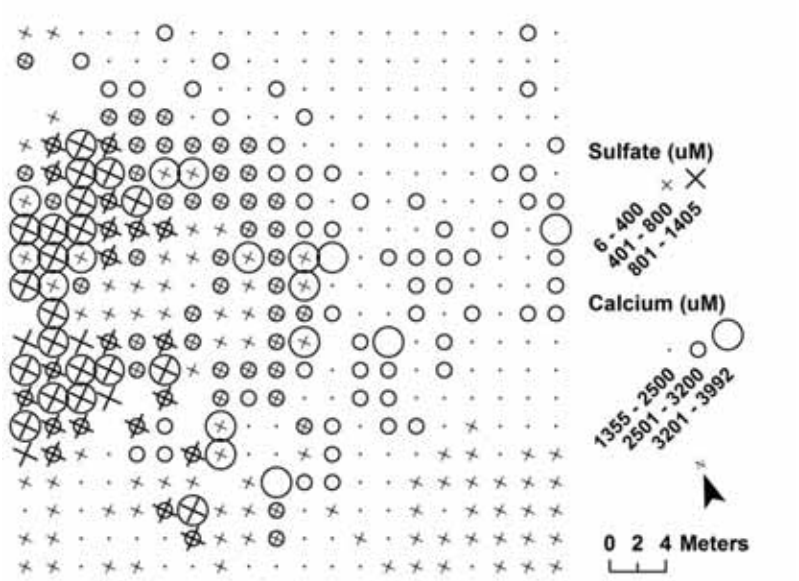
Porewater calcium and sulfate were heterogeneous and spatially independent (Figure 4-3a), consistent with the nested groundwater flows to the site (Boomer and Bedford 2008b) and the mixture of underlying limestone (calcium carbonate) and anhydrite/gypsum ($CaSO_4$/ $CaSO_4 \cdot 2 H_20$). Porewater calcium was above the 60^{th} percentile of previously studied fens in New York State (Godwin et al. 2002) and comparable with calcareous fens studied in Europe (Lucassen et

al. 2004, Horsak et al. 2012). Porewater sulfate spanned most of the range of previously studied fens in New York State but was usually above the 90th percentile of those previously studied sites (Godwin et al. 2002), and was somewhat lower than for previous studies in the Netherlands (Lucassen et al. 2002, Lucassen et al. 2005). Almost all sulfate values were greater than 20 uM. Higher sulfate concentrations of 400 uM or greater were more abundant in the southwestern half of the grid, and the highest sulfate concentrations in the 800-1400 uM range were mostly prevalent west of the biggest interior woody island.

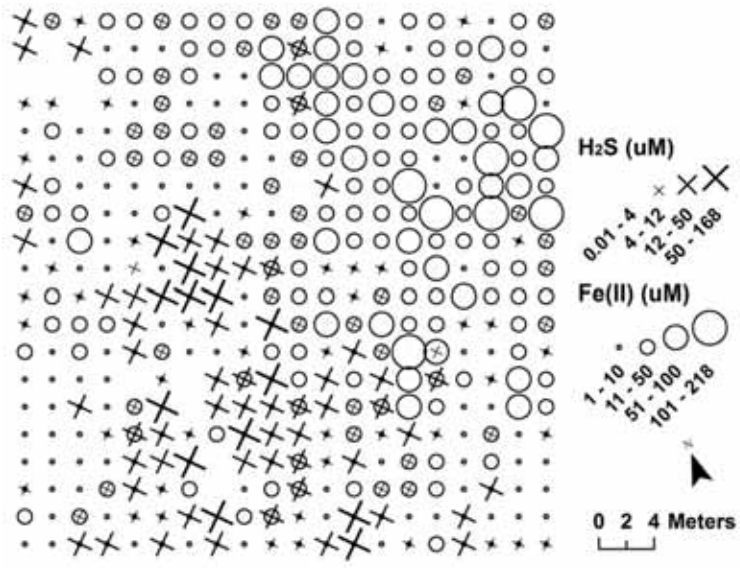
Sulfate was not a good predictor of sulfide, as expected from numerous literature studies of sulfate reduction and sulfide oxidation showing that carbon quality, temperature and other biological influences are important (Wieder et al. 1990). Although sulfate supply may have helped set an upper limit on sulfide accumulation from 0-500 uM sulfate, at 800 uM sulfate and greater the concentrations of sulfide were consistently low; the cause of this is unknown. The highest sulfide concentrations were at 150-800 uM sulfate, but there were also many very low sulfide values at this same range of sulfate.

Concentrations of H_2S, the most toxic form of sulfide, were highly heterogeneous, ranging from 0-168 uM (mean=8 uM) (Figure 4-3b), as were concentrations of HS^- , which ranged from 0-210 uM. In half of the locations, 35-50% of sulfide was in the form of H_2S, with the remainder as HS^-. The pH was not high enough for there to be any ecologically significant quantity of S^{2-}. Most H_2S in excess of 12 uM (and up to 168 uM) was in or near the central woody island and at the margins of the standing water to the south. However, there were H_2S values of 4 uM or more scattered throughout the plot, including in areas with sulfate 50 uM or less.

The porewater chemistry variables that I expected to be most closely related to sulfide



(a)



(b)

Figure 4-3. (a) Sulfate and calcium, which are both delivered by groundwater, showed a spatially heterogeneous summer pattern. Sulfate to calcium ratios vary, as expected given the mixture of underlying calcium carbonate and calcium sulfate. Data from Junius Pond fen, NY. (b) H_2S and Fe(II), the reduced forms of sulfur and iron, were highly heterogeneous in the summer in Junius Pond fen, NY. Concentrations of these two reduced species reflect numerous biogeochemical reactions, including those highlighted in my conceptual model in Figure 4-1. The higher concentrations of H_2S and Fe(II) occurred in spatially distinct locations, likely reflecting, in part, co-precipitation as FeS or FeS_2 that removes them both from solution. The less toxic form of sulfide, HS^-, is not displayed.

were themselves highly variable. Ferrous iron (Fe(II)), like sulfide a reduced chemical species, was highly heterogeneous (Figure 4-3b), ranging from 0.2-217.5 uM (mean=19.7). Higher concentrations of Fe(II) occurred in spatially distinct locations from H_2S, likely reflecting in part co-precipitation as FeS or FeS_2 that removes them both from solution. Resin phosphorus, expected to reflect in part phosphate released from ferric iron by sulfide, was also highly heterogeneous, ranging from 0-273 umol mo^{-1} $10cm^{-2}$ (mean=10). H_2S was not positively correlated with phosphorus availability, contrary to expectations from the conceptual biogeochemical model in which sulfide chemically reduces Fe(III) to Fe(II) and thereby releases iron-bound phosphorus (Figure 4-1a). This is also contrary to previous data collected at several other rich fens (Bergen and Belle School) of New York State (Simkin et al., unpublished data).

Descriptive statistics of other commonly measured porewater chemistry variables are provided here for comparison. The average pH was circumneutral (7.10) and the range of pH was low (7.0-7.2 from 1^{st} to 3^{rd} quartile) and showed little patchiness, as expected for a rich fen receiving large inputs of neutralizing carbonate. Temperature corrected conductivity averaged 608 uS and ranged from 258 – 914 uS. The range of sodium (4.1-20.3 mg/L) and chloride (3.6-21.3 mg/L) was relatively low, in contrast with the wide range expected in estuaries where sulfate and chloride covary with the fraction of water contributed by seawater versus freshwater. In this "freshwater" wetland the connection between sulfate and chloride is separated. Total dissolved nitrogen (TDN) was higher than expected, ranging from 5 – 66 uM (mean=21).

PLANT COMMUNITY CHARACTERISTICS AND PATTERNS

At Junius there were a total of 44 species, including 16 dicots, 14 monocots, 10 mosses, 1

gymnosperm, 1 fern, 1 alga, and 1 liverwort. Species density per 100 cm^2 plot averaged four and was as high as 11, with up to five monocot species, five dicot species, or five moss species in a single 100 cm^2 plot. *Campylium stellatum* was the moss species that occurred most frequently (239 out of 400 locations) and had the greatest percent cover (median=10%, mean=32.16%, range=0-100%). *Eleocharis rostellata* was the monocot species that occurred most frequently (269 out of 400 locations) and had the greatest percent cover (median=6%, mean=11.98%, range=0-80%), even though it is rare in the landscape outside of marl fens and rich graminoid fens (Olivero 2001, Wesley et al. 2008). *Dasiphora floribunda* was the dicot species that occurred most frequently (130 out of 400 locations) and had the greatest percent cover (median=0%, mean=4.46%, range=0-85%). In contrast to many other wetland and terrestrial systems, total species density was positively correlated with total percent cover ($r^2 = 0.26$, $P < 0.0001$), driven in part by the substantial number of low-species locations with total cover less than 50%.

MODEL COMPARISONS OF VEGETATION IN RELATION TO POREWATER CHEMISTRY

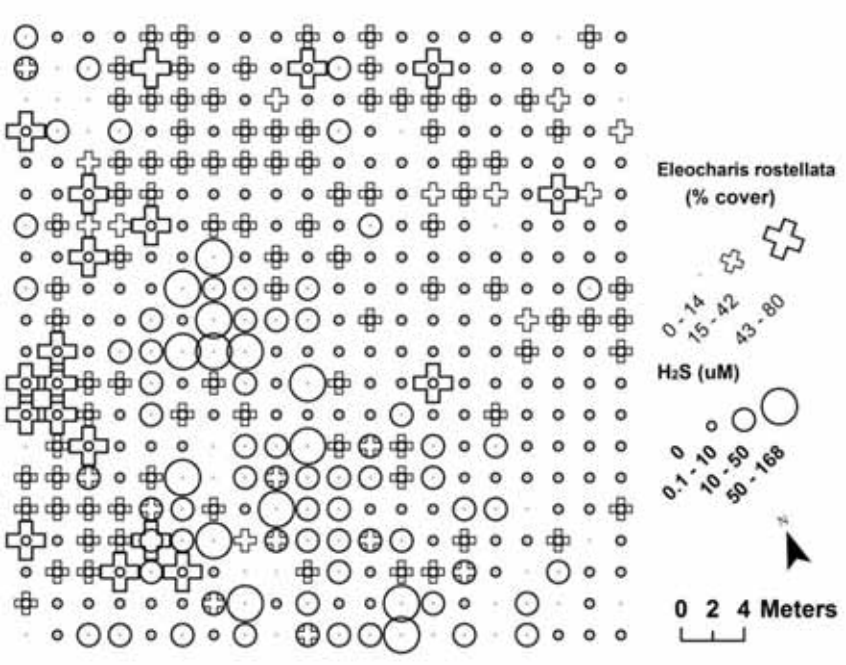
Model comparisons using AICc values showed that the combination of presence and percent cover of monocot, dicot, and moss functional groups was explained better by the three models containing sulfide than by any of the other eight models which did not. The best model of total plant cover included sulfide with a negative parameter estimate and calcium with a positive parameter estimate. More specifically, monocots and mosses were heavily supported by the sulfide plus calcium model (Hypothesis 4 in Table 4-1, Table 4-2), while dicots were most

supported by the sulfide toxicity model (Hypothesis 1 in Table 4-1, Table 4-2). Average parameter estimates show that sulfide had a negative value in the models of moss and monocot cover. The role of sulfide was more equivocal for dicot cover, as the binomial parameter estimate was very negative but the conditional Gaussian estimate was small and actually slightly positive. Calcium had a positive value in moss and monocot cover models, but a slightly negative value in dicot models.

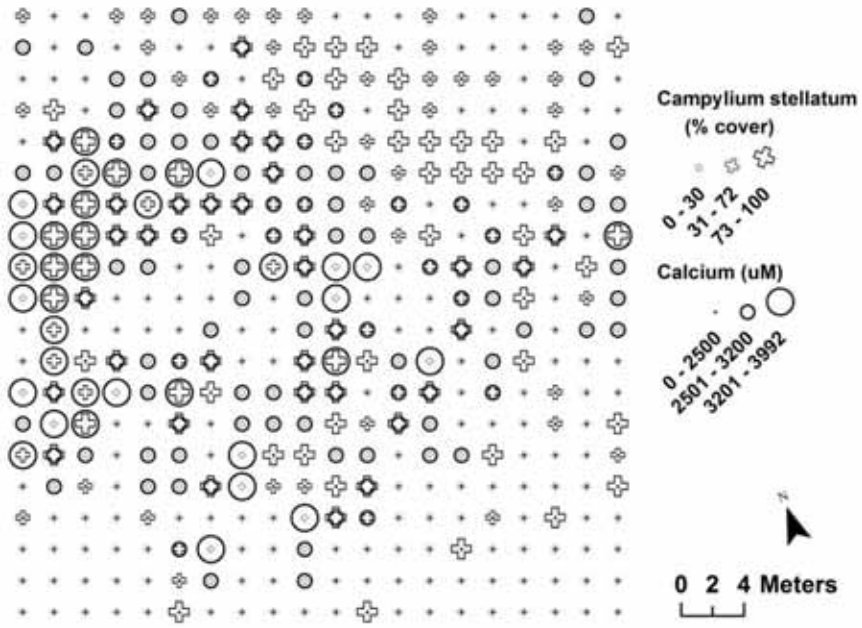
For the three most frequently occurring species in this fen, (*Eleocharis rostellata* (Figure 4-4a), *Campylium stellatum* (Figure 4-4b), and *Cladium mariscoides*), the most important cover models included sulfide and either phosphorus or calcium (Hypothesis 3 or 4 in Table 4-1, Table 4-2). Sulfide parameter estimates were negative and calcium parameter estimates were positive for these three species. Contrary to expectations, phosphorus parameter estimates were negative. Note that outside of fens these three species occur only infrequently.

Dasiphora floribunda, the fourth most frequently occurring species and the most frequent of all the dicot species, was uniquely ambiguous in that there was support for the sulfide only cover model (Hypothesis 1 in Table 4-1), yet there were divergent signs for the sulfide parameter in the binomial and percent cover components of the model (Table 4-2). Specifically, *D. floribunda* was less likely to be present where sulfide was high, but if it was present then it had higher cover where sulfide was high, consistent with potentially different mechanisms for establishment versus dominance.

For the remainder of the modeled species, which all occurred in 5-20% of the plots, there was a greater diversity of cover models with a good fit to the data (Hypothesis 1, 4, 5, or 7 in Table 4-1, Table 4-2). Models containing sulfide continued to be important for some species, such as *Carex aquatilis, Sarracenia purpurea*, *Dichantelium acuminatum*, and *Plagiothecium*



(a)



(b)

Figure 4-4. (a) Cover of *Eleocharis rostellata*, the most frequently occurring species in Junius Pond fen, NY, is best explained by models that include porewater sulfide with a negative coefficient. (b) Cover of *Campylium stellatum*, the most frequently occurring moss species in Junius Pond fen, NY, is best explained in models that include porewater calcium with a positive coefficient.

Table 4-2. Model importance and parameters of vegetation cover models. Each row represents a single plant functional group or species, displayed in descending order according to frequency of occurrence at Junius Pond fen, NY. Each of the 11 columns in the center of the table is for a separate model representing each of the hypotheses/models in Table 4-1, with the decimal value indicating the model weight/importance derived from ΔAICc values of the specific model and all the other models in the model set. For each species or functional group, the model with the greatest weight is highlighted in bold and the sum of the weights of the 11 models is 1. The right-hand columns of the table are average parameter estimates, weighted by model weights. Only parameter estimates for the three variables (sulfide, phosphorus, and calcium) that were expected to be the most important *a priori* are presented. Separate parameter estimates for both parts of each model are provided: binomial (presence/absence only) and Gaussian (percent cover only where cover > 0). Negative parameter estimates are highlighted in bold.

			Importance of each numbered hypothesis derived from dAICc scores (each species sums to 1)												Parameter values (x10000) averaged across model set					
			1	2	3	4	5	6	7	8	9	10	11							
			HS	P	HS_P	HS_CA	CA	FE2	FE2_SO4	SO4_N	N_P	CA_FE2_SO4_N	mean	HS (binom)	HS	P (binom)	P	CA (binom)	CA
Functional groups	Freq	CODE																		
Mono+Dicot+Moss+other	380	TOTL	0.01	0.00	0.01	**0.97**	0.01	0.00	0.00	0.00	0.00	0.00	0.00		**-6775**		0		8
Monocots	351	MONO	0.00	0.00	0.00	**1.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	382	**-6270**	**-45**	0	13	9
Moss	312	MOSS	0.17	0.00	0.03	**0.78**	0.01	0.00	0.00	0.00	0.00	0.00	0.00	**-5228**	**-2557**	-8	2	0	6
Dicots	249	DICO	**0.43**	0.00	0.19	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**-6000**	12	0	17	0	**-2**
Species	Freq	CODE																		
Eleocharis rostellata	254	ELRO	0.00	0.00	**1.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**-8860**	**-1079**	**-1706**	**-3**	0	2
Campylium stellatum	232	CAST	0.00	0.00	0.00	**0.95**	0.05	0.00	0.00	0.00	0.00	0.00	0.00	**-2338**	**-4877**	**-114**	0	3	13
Cladium mariscoides	148	CLMA	0.00	0.28	**0.42**	0.16	0.08	0.00	0.00	0.00	0.04	0.03	0.00	**-83**	**-1034**	**-188**	**-36**	4	0
Dasiphora floribunda	127	DAFL	**0.42**	0.02	0.05	0.20	0.07	0.02	0.02	0.03	0.00	0.02	0.15	**-2221**	606	2	**-2**	2	**-1**
Scorpidium scorpioides	70	SCSC	0.00	0.00	0.00	0.00	0.00	0.00	**0.43**	0.43	0.00	0.13	0.00	0	**-1279**	0	**-20**	6	1
Carex aquatilis	71	CAAQ	0.04	0.00	0.01	**0.77**	0.10	0.07	0.01	0.00	0.00	0.01	0.00	4736	**-30**	1	22	**-6**	**-1**
Drosera rotundifolia	61	DRRO	0.01	0.01	0.00	0.00	0.01	0.01	**0.78**	0.13	0.00	0.01	0.04	**-98**	155	6	2	0	0
Thelia hirtella	60	THHI	0.00	0.00	0.00	0.00	0.00	0.00	**0.86**	0.12	0.00	0.02	0.00	0	**-333**	0	22	2	0
Thuja occidentalis	48	THOC	0.00	0.00	0.00	0.00	0.00	0.00	**0.54**	0.45	0.00	0.00	0.00	0	4504	0	5	0	0
Mentha arvensis	44	MEAR	0.00	0.00	0.00	0.00	0.00	0.06	**0.85**	0.06	0.00	0.02	0.00	**-4**	**-295**	0	0	**-4**	**-3**
Sarracenia purpurea	39	SAPU	**0.61**	0.02	0.07	0.14	0.03	0.02	0.00	0.00	0.00	0.00	0.12	**-3544**	**-4088**	16	-4	1	**-1**
Fissidens adianthoides	39	FIAD	0.02	0.06	0.01	0.00	0.03	0.02	**0.39**	0.32	0.00	0.01	0.15	152	96	2	13	0	**-3**
Sphagnum centrale	37	SPCE	0.00	0.00	0.00	0.00	0.00	**0.45**	0.14	0.00	0.00	0.40	0.00	0	1735	0	8	**-16**	4
Dichanthelium acuminatum	35	DIAC	0.10	0.01	0.33	**0.54**	0.01	0.00	0.00	0.00	0.00	0.00	0.00	**-6465**	**-2448**	**-748**	**-9**	0	**-3**
Aneura pinguis	27	ANPI	0.01	0.00	0.00	0.00	0.00	0.34	**0.44**	0.18	0.00	0.00	0.03	1	1021	0	**-19**	0	**-1**
Morella penslyvanica	22	MOPE	0.01	0.01	0.00	0.06	**0.86**	0.02	0.00	0.00	0.00	0.00	0.04	1725	**-1953**	3	1	**-13**	**-3**
Plagiothecium laetum	21	PLLA	**0.71**	0.04	0.04	0.04	0.01	0.02	0.00	0.00	0.00	0.00	0.13	5586	2087	**-155**	**-191**	0	**-1**

Table 4-3. Model importance and parameters of vegetation richness, height, and structure. Each row represents the species density of a single plant functional group or species at Junius Pond fen, NY. Each of the 11 columns in the center of the table is for a separate model representing each of the hypotheses from the introduction, with the numeric value indicating the model weight/importance derived from ΔAICc values of the specific model and all the other models in the model set. For each species or functional group, the model with the greatest weight is highlighted in bold and the sum of the weights of the 11 models is 1. The right-hand columns of the table are average parameter estimates, weighted by model weights. Negative parameter estimates are highlighted in bold. The models for % bare, % water, and % bare include both binomial and continuous components, while the other indices are based entirely on continuous models.

	Importance of each numbered hypothesis derived from dAICc scores (each species sums to 1)											Parameter values (x10000) averaged across model set					
	1	2	3	4	5	6	7	8	9	10	11						
Index	HS	P	HS_P	HS_CA	CA	FE2	FE2_SO4	SO4_N	N_P	CA_FE2_SO4_N	mean	HS_wt	P_wt	CA_wt	FE2_wt	SO4_wt	N_wt
nonvasc_spp	0.00	0.00	0.00	0.00	0.00	0.00	0.43	**0.43**	0.00	0.13	0.00	0.80	**-0.13**	0.17	0.44	**-6.37**	4.33
mono_spp	0.00	0.00	0.00	0.03	0.03	0.00	0.00	0.00	0.00	**0.94**	0.00	**-19.05**	**-0.40**	4.31	**-2.82**	**-4.51**	**-103.65**
dico_spp	**0.50**	0.00	0.31	0.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**-2528.07**	6.87	0.06	0	0	0
ELRO_cm	0.16	0.01	0.16	**0.53**	0.02	0.00	0.00	0.04	0.05	0.02	0.01	**-14732.48**	432.14	**-8.84**	2.88	**-0.58**	**-113.18**
height_cm	0.00	0.00	0.00	0.26	**0.49**	0.00	0.00	0.00	0.00	0.24	0.00	**-2597.77**	0.14	**-62.11**	**-66.93**	0.75	**-455.62**
% bare	0.00	0.00	**1.00**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	124917.59	**-475.52**	**-5.25**	0	0	0
% water	0.00	0.00	**0.86**	0.00	0.00	0.00	0.05	0.03	0.00	0.06	0.00	233754.45	**-3811.29**	3.18			
% litter	0.01	0.00	**0.79**	0.09	0.00	0.00	0.00	0.00	0.07	0.04	0.00	**-81290.87**	1175.16	**-27.36**			

laetum, but to my surprise the Fe(II) plus sulfate model was the most important for *Scorpidium scorpioides*, *Drosera rotundifolia*, *Thelia hirtella*, *Thuja occidentalis*, *Mentha arvensis*, *Fissidens adianthoides*, and *Aneura pinguis*. The Fe(II) plus sulfate model (Hypothesis 7 in Table 4-1) was not one that I had expected to be important *a priori*, but rather was a model that was included largely to balance the model set. Fe(II) alone (Hypothesis 6 in Table 4-1) was the most important cover model for *Sphagnum centrale* and calcium alone (Hypothesis 5 in Table 4-1) was the most important cover model for *Morella pennslyvanica*. Among the species where cover models including sulfide were important, sulfide parameter estimates were actually positive for *Plagiothecium laetum* and for the binomial component of *Carex aquatilis*. For the less frequent species where calcium was important in cover models, namely *Carex aquatilis*, *Dichanthelium acuminatum*, and *Morella pennslyvanica*, the calcium parameter estimate was negative.

The species with the most model uncertainty are arguably *Dasiphora floribunda*, *Sarracenia purpurea*, *Fissidens adianthoides*, and *Plagiothecium laetum*, given that the simple model of mean cover (Hypothesis 11 in Table 4-1) without any environmental predictors has a model weight of 12% or more for these species. Of these, all but *Fissidens adianthoides* have sulfide-only as the model with the greatest weight. Model certainty may be better in species with models that couple sulfide with other environmental variables compared to those where only sulfide is important.

Dicot species density (richness / 100 cm^2) was best explained by models including sulfide (Hypotheses 1,3, and 4 in Table 4-1, Table 4-3), while monocot and moss species density was best explained by models including sulfate, Fe(II), and nitrogen (Hypotheses 7,8, and 10 in Table 4-1, Table 4-3). More specifically, the sulfide parameter estimate for dicot species density was

negative while phosphorus and calcium were positive. Both monocot and moss species density had negative sulfate parameter estimates. Monocot species density had negative parameter estimates for Fe(II) and nitrogen, while mosses had positive estimates for these same parameters.

As a partial replacement for biomass measurements, which I deemed too destructive for this sensitive site, results of analyses of vegetation height and percentage bare ground are presented here (Table 4-3) to complement vegetation cover analyses. The height of *Eleocharis rostellata* was best explained by models containing sulfide, in combination with calcium and phosphorus, with sulfide a very negative parameter, calcium another negative parameter, and phosphorus a positive parameter. The overall maximum vegetation height, which was not *Eleocharis rostellata* in about 1/3 of all locations, was best explained by a calcium-only model, and to a lesser extent by sulfide and every other environmental variable except phosphorus. As with *Eleocharis rostellata* height, parameters for calcium and sulfide were negative, as were Fe(II) and nitrogen. The percentage of area covered by bare ground and standing water was best explained by the sulfide plus phosphorus model, with a positive parameter for sulfide and a negative parameter for phosphorus. The percentage of area covered by litter was also best explained by the sulfide plus phosphorus model, but the signs of the parameters were reversed. Collectively, these latter three indices are all consistent with less live or dead biomass when sulfide is high, and more live or dead biomass when phosphorus is high.

MULTIVARIATE ANALYSES

Non-metric multidimensional scaling (NMS) ordination of square-root transformed cover data of plots in species space indicated that linear vectors of calcium (uM), phosphorus (umol

mo^{-1} $10cm^{-2}$), total ($H_2S + HS^-$) sulfide (uM), Fe(II) (uM), and temperature corrected conductivity (uS/cm) were significantly correlated ($P < 0.005$) with the NMS axes, but sulfate and TDN were not ($P > 0.4$). Specifically, calcium, conductivity, and Fe(II) were negatively correlated with the first NMS axis and positively correlated with the second axis, phosphorus positively correlated with the first NMS axis, and sulfide negatively correlated with the second NMS axis. Nonlinear fitting of thin plate splines in a General Additive Model (GAM) framework, using ordisurf function in R vegan package (Oksanen et al. 2011) suggested that the sulfide gradient mostly exists only where calcium is high (Figure 4-5).

Discussion

POREWATER HETEROGENEITY

I found an extraordinary degree of heterogeneity in porewater chemistry, including in groundwater-delivered ions, reduced species toxins, and nutrients. Coarse-scale heterogeneity of porewater geochemistry within fens has been measured previously (Koretsky et al. 2007), but has rarely if ever been measured at a scale capable of capturing variability in two dimensions across distances of several meters to dozens of meters and with a sample size (400) adequate to span the range of abundances of infrequently occurring species in a diverse rich fen plant community. Spatial heterogeneity of reduced chemical species in porewater was expected based on previous work indicating groundwater delivery along nested groundwater flowpaths (Boomer and Bedford 2008b), but empirical confirmation at this scale and intensity has not occurred until

now, likely due in part to the extra steps needed to capture redox-sensitive parameters such as sulfide and Fe(II).

OVERVIEW OF PLANT RESPONSE TO POREWATER HETEROGENEITY

I had hypothesized that sulfide's direct toxicity and indirect mobilization of phosphorus would produce clear and distinct species-specific differences in performance. I anticipated that fast-growing species with high sulfide tolerance would benefit at the expense of slower-growing species with low sulfide tolerance. As expected, direct sulfide toxicity was important in determining plant species composition and structure. Contrary to expectations, indirect sulfide mobilization of phosphorus was not readily relatable to sulfide, and neither phosphorus nor nitrogen greatly influenced plant species composition. Sulfide did frequently interact with calcium to influence plant species composition. Each of these relationships between vegetation and water chemistry is addressed in greater detail below.

SULFIDE INFLUENCE ON PLANT COMPOSITION

Hydrogen sulfide reduced total plant cover, reduced the cover of the three most frequently occurring species (including two monocot species and a moss species), reduced dicot species density, reduced plant height, reduced litter accumulation, and increased the percentage of bare ground, consistent with sulfide interfering with the energy budget of plants. However, hydrogen sulfide was unimportant in models of moss or monocot species density and in cover models of most infrequently occurring species. Furthermore, as will be discussed later, the

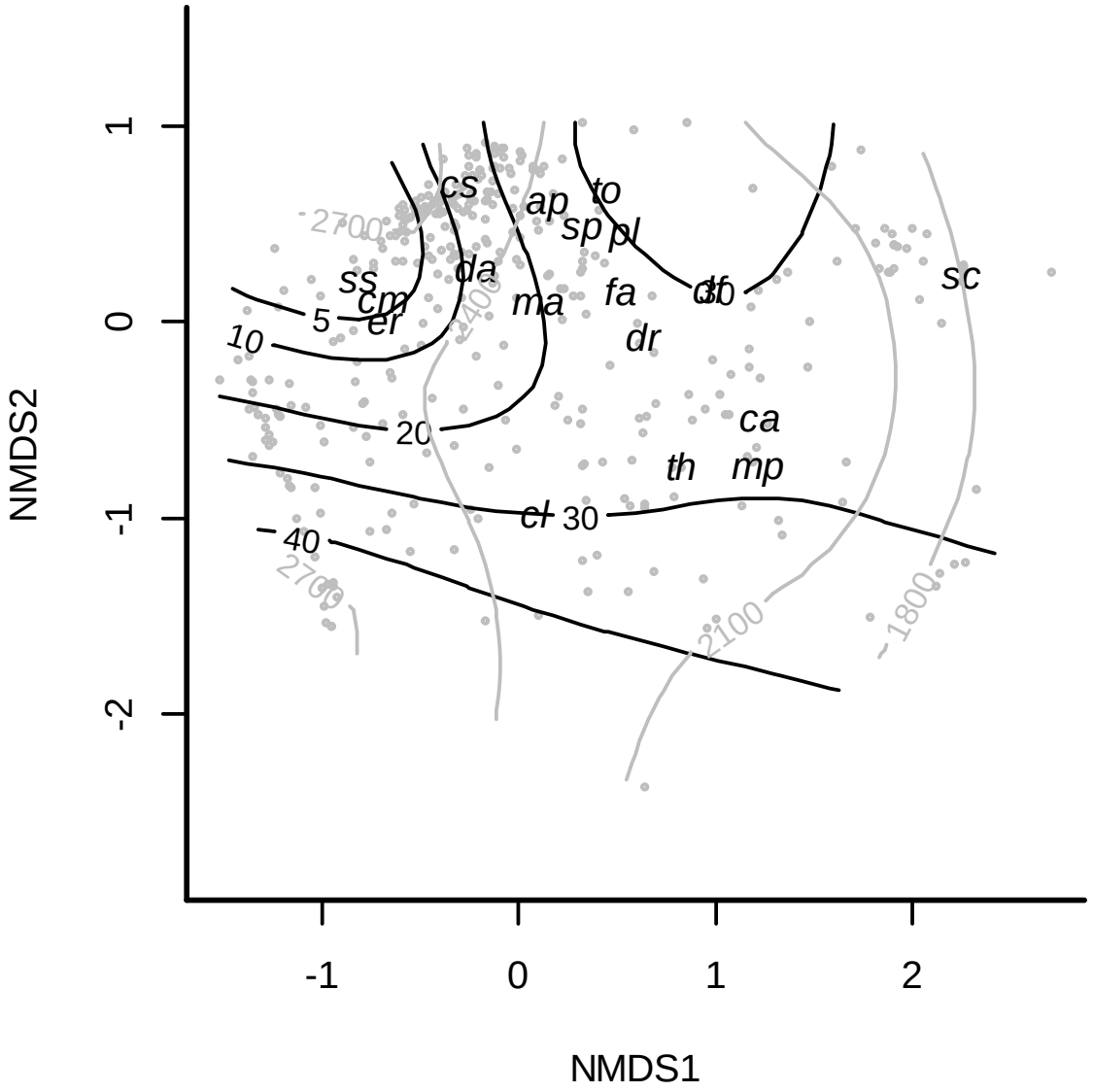


Figure 4-5. Non-metric multidimensional scaling (NMS) ordination of square-root transformed cover data of plots (gray dots) in species space. Species correlations with axes are shown as lowercase two character species codes in italics, with the first letter indicating the first letter of the genus and the second letter indicating the first letter of the species. Contours indicate environmental variables fit to ordination using general additive modeling, with gray contours indicating calcium (uM) and black contours indicating total ($H_2S + HS^-$) sulfide (uM). Sulfide contours are nonlinear, demonstrating a steep gradient at calcium above 2400 uM.

presence/absence and abundance components of cover models for *D. floribunda*, *C. aquatilis*, and *M. penslyvanicum* were inconsistent. As a whole, hydrogen sulfide had a negative influence on plants, but this influence was surprisingly subtle given the highly phytotoxic nature of hydrogen sulfide. One possibility, adapting a phrase from the competition literature, is that I am seeing the ghosts of toxicity past. All species that were highly intolerant of sulfide may have been filtered out over the course of the last one hundred years or more. This is in contrast to restoration efforts in the Netherlands where sulfate-rich water from the surrounding landscape was introduced *de novo* to rewet wetlands and the effects could be observed in real time over the course of months or several years. In the present study there have presumably been groundwater inputs of sulfate for one hundred years or more, allowing ample time for extremely strong selective pressure on plants to evolve multiple sulfide avoidance or tolerance mechanisms, such as physiological sequestration of sulfide into non-toxic reduced sulfur compounds such as glutathione, or development of root barriers to entry of sulfide.

The inconsistent response of the shrubby species *D. floribunda* may arise from one or more of several hypothetical possibilities. *D. floribunda* may have differential sensitivity to sulfide at different life-stages, with the seedling stage sensitive to sulfide, as reflected in the negative binomial coefficient, and the adult stage insensitive to sulfide or at least sufficiently less sensitive than competitors that they are able to pre-empt space even though sulfide is high. Alternatively, aboveground cover of *D. floribunda* adults may not always indicate an underlying live root system, as the stem may sprawl laterally and root where there is less sulfide. This latter alternative is a distinct possibility since sulfide is so heterogeneous and therefore the distance to the nearest low-sulfide microsite may be quite small.

In contrast to *D. floribunda*, I can speculate that the two species *C. aquatilis* and *M.*

penslyvanicum may be tolerant of sulfide as seedlings but then become less tolerant as adults. Alternatively, they may occur more frequently in higher sulfide locations simply because there is a comparative void of other species that can persist there. Stated another way, these two species might not preferentially establish where there is higher sulfide, but may instead be incrementally less sensitive to sulfide than other species that have competitively displaced them from lower sulfide locations. Future experimental work in mesocosms or the greenhouse is required to address this possibility of differential plant responses at different life stages.

My *a priori* expectation was that sulfide would have a limited direct influence on mosses since mosses inhabit the surface zone, presumably above much of the sulfide, and that indirectly mosses could benefit where sulfide was high if rooted plants were inhibited and therefore left space and light for the mosses. There was indeed little role for sulfide or phosphorus or calcium in the occurrence and abundance of most nonvascular species, with the large exception that the most frequently occurring non-vascular species, the brown moss *C. stellatum*, was best explained by models that incorporated sulfide with a negative coefficient. Perhaps the high-sulfide zone extends closer to the surface than I assumed.

Generalizing the influence of sulfide on monocot and dicot functional groups, it is possible that sulfide has a stronger negative influence on presence/absence in dicots and a stronger negative influence on abundance in monocots. Within dicots, I had initially assumed that woody species would be more deeply rooted and therefore more sensitive to sulfide than herbaceous species. I do not, however, have rooting depth data to lend support to this assumption. As discussed earlier, the woody species *D. floribunda* and *M. penslyvanica* both had a complex relationship with sulfide, speculatively perhaps as a consequence of differential sensitivity at different life stages. It is also important to note that my methodology relating a

point sample of water chemistry to a small 10-cm by 10-cm plot is best suited to small-statured herbs, sedges and mosses, whereas large woody species may have rooting zones extending over many square meters with a much wider range of water chemistry than can be represented in a point sample.

Possible explanations for the more modest than expected influence of porewater sulfide on plant community composition might include: (1) sulfide concentrations in bulk porewater are likely higher than the concentrations that plants are exposed to in the rhizosphere due to radial oxygen loss, sulfide oxidation by microbes in the rhizosphere, and precipitation of sulfide with iron or other metals. (2) Root membrane barriers to H_2S and HS^- entry may be a more universal mechanism of sulfide avoidance than expected. Finally, (3) internal plant mechanisms to detoxify sulfide may be stronger than expected, and there may be physiological plasticity to variable sulfide exposure and/or fixed physiological tolerance mechanisms that are energetically cheap. There appears to be one or more sulfide tolerance or avoidance mechanisms that work reasonably well for the species that occur in this fen, thereby reducing differential effects on species. Instead of highly sulfide-tolerant species benefitting from higher sulfide at the expense of sulfide-intolerant species, it appears that most or all species are mildly sulfide-intolerant, ultimately benefiting the least abundant species.

VERY MODEST RELATIONSHIP OF NUTRIENTS TO VEGETATION

I was able to detect at best only a modest vegetation relationship with phosphorus and no relationship with nitrogen. The dominant plant species, *Eleocharis rostellata*, and to a much lesser extent all plant species, was taller when there was more phosphorus. There was also an

additional positive association of phosphorus with litter cover. In contrast, however, live *Eleocharis rostellata* cover had a negative association with resin phosphorus. The relationship of phosphorus to vegetation was therefore ambiguous. The other measured nutrient nitrogen was uniformly absent from the best models of total plant cover, litter, and plant height. Overall, my proxies for nutrient availability were not strongly related to plant abundance, contrary to expectations.

There are several possible explanations for the modest relationship of phosphorus to plant cover, both methodological and ecological. I chose resin phosphorus accumulated over a period of one month as my index of phosphorus availability, which was preferable to simple aqueous phosphate that was consistently below detection limit. Resin phosphorus has been shown to correlate well with tissue concentrations of phosphorus in uplands (Qian and Schoenau 2002). In my own field tests in other fens (Simkin et al., unpublished data) I found a good correlation between resin phosphorus and plant tissue phosphorus. However, in laboratory loading tests I found that high loadings of other anions such as sulfate reduced phosphate recovery (Simkin et al., unpublished data), raising the possibility that patterns of phosphorus release from the soil and adsorption to the resin surface may have been partially masked by subsequent desorption of phosphate by sulfate and other anions delivered via groundwater. Furthermore, in several other rich fens there were ancillary data available to indicate that under some circumstances resin phosphorus is a better indicator of plant phosphorus uptake than of phosphorus supply (Crowley and Bedford 2011). Additional follow-up work in larger fens with fewer endangered species and therefore fewer concerns about extensive destructive biomass sampling and application of ^{32}P isotopic tracers could address these methodological issues in more detail.

Previous work has indicated that phosphorus limitation or phosphorus and nitrogen co-

limitation frequently occur in rich fens of North America (Bedford et al. 1999) and detailed hydrological and geochemical work at this site in particular suggested that sulfide mobilization of iron-bound phosphorus should occur (Boomer and Bedford 2008b). At this site sulfide may have been high enough to reduce most iron and prevent phosphorus precipitation with iron, and phosphorus and pH may have been low enough to prevent phosphorus precipitation with calcium in the marl area. However, since total soil inorganic phosphorus measured at this site was low (Boomer and Bedford 2008a), then sulfide mobilization of phosphorus may not have been as relevant to plants at this site compared to other sites with higher soil inorganic phosphorus pools.

CALCIUM RELATED TO PLANT COVER

Fen indicator plants are usually considered to be calciphytes, and in the upper-Midwestern and northeastern United States most rich fens are extremely calcium-rich. However, my *a priori* assumption was that calcium was simply an indicator of groundwater supply and therefore a proxy for stable water table elevations, water temperature, circumneutral pH, and a wide variety of anions, including terminal electron acceptors such as sulfate. However, in the end, my results affirm previous research pointing to the importance of calcium to fen plant species. Even within a single rich fen with reasonably homogenous water table and water temperature and a limited pH range, calcium was associated with almost every aspect of the fen vegetation. Cover of every plant functional group and many species, both frequently and infrequently occurring ones, was associated with calcium, as was plant height. Calcium was highly correlated with the primary ordination axis of plant community matrix dissimilarities. Looking collectively at ordinations and the signs of coefficients that appear in the best models,

some species were clearly highly calciphilic (*C. stellatum* and *C. mariscoides*) while other species were less calciphilic (*C. aquatilis*, *M. penslyvanica*). On the basis of plant height, even the dominant species *E. rostellata* appears to be less calciphilic. Using single lines of evidence additional species may be classified according to their degree of calciphily.

IMPORTANCE OF COMBINED ABUNDANCE AND PRESENCE/ABSENCE DATA

Two-part models incorporating both quantitative percent cover values and binomial presence/absence data should probably be used in plant biogeography studies more frequently than is currently the case. The two-part modeling approach utilized in this study was originally utilized to deal with the problem of zero-inflated data that is common in vegetation studies, but other advantages of this approach also emerged. First, two-part models make use of all available data from rare species, avoiding the loss of information content that occurs when stripping away abundance data from rare species and converting to simple presence/absence data. Second, two-part models could allow for the incorporation of separate environmental variables in the binomial and cover components of the models based on *a priori* knowledge of life history traits specific to seedling and adult stages. The latter benefit encourages a richer array of hypotheses about plant-environment relationships.

IMPLICATIONS FOR OTHER ECOSYSTEMS

I suspect that these inland studies of sulfide and plants will have relevance in high sulfate coastal systems as well. For example, some coastal species thought to be intolerant of salinity

may in fact be more intolerant of sulfide, and conversely some species thought to be intolerant of sulfide may be more intolerant of salinity. At the lower end of the sulfur abundance spectrum, I suspect that some plant-sulfur relations in bogs could be informed by my work in fens. For example, *Sarracenia purpurens* occurs in some fens despite being more typically associated with bogs. If it is relatively intolerant of sulfide in fens, then changes in atmospheric deposition of sulfur may have influenced its distribution.

CONCLUSIONS

The relationship of phytotoxic sulfide to plant community structure in a rich fen ecosystem that was identified here augments prior knowledge of the influence of calcium on vegetation. This work highlights the more general fact that in ecosystems such as rich fens with high plant diversity there are almost inevitably important but as-of-yet unnoticed environmental gradients waiting to be discovered, even though such ecosystems have already been thoroughly studied. The present study began with a traditional environmental gradient framework and then elaborated upon that framework by extending the scope of study to additional chemical constituents (in this case redox-senstive chemistry) and to a finer (2-m resolution) spatial scale. Similar extensions of environmental gradient frameworks to new chemical and spatial dimensions may yield insights on plant community structure in other species-diverse ecosystems.

REFERENCES

A.P.H.A. 2005. 4500 - S^{2-} (SULFIDE). Page 4.170 to 174.183 *in* A. D. Eaton, L. S. Clesceri, E. W. Rice, and A. E. Greenberg, editors. Standard methods for the examination of water & wastewater. American Public Health Association, Washington, DC.

Almendinger, J. E. and J. H. Leete. 1998. Peat characteristics and groundwater geochemistry of calcareous fens in the Minnesota River Basin, USA. Biogeochemistry 43:17-41.

Anderson, D. R. 2008. Model based inference in the life sciences: a primer on evidence. Springer, New York.

Bagarinao, T. 1992. Sulfide as an environmental-factor and toxicant - Tolerance and adaptations in aquatic organisms. Aquatic Toxicology 24:21-62.

Becana, M., J. F. Moran, and I. Iturbe-Ormaetxe. 1998. Iron-dependent oxygen free radical generation in plants subjected to environmental stress: toxicity and antioxidant protection. Plant and Soil 201:137-147.

Bedford, B. L., M. R. Walbridge, and A. Aldous. 1999. Patterns in nutrient availability and plant diversity of temperate North American wetlands. Ecology 80:2151-2169.

Boomer, K. and B. Bedford. 2008a. Groundwater-induced redox-gradients control soil properties and phosphorus availability across four headwater wetlands, New York, USA. Biogeochemistry 90:259-274.

Boomer, K. M. B. and B. L. Bedford. 2008b. Influence of nested groundwater systems on reduction-oxidation and alkalinity gradients with implications for plant nutrient availability in four New York fens. Journal of Hydrology 351:107-125.

Bridgham, S. D., J. Pastor, J. A. Janssens, C. Chapin, and T. J. Malterer. 1996. Multiple limiting gradients in peatlands: A call for a new paradigm. Wetlands 16:45-65.

Cadwell, D. H., G. G. Connally, P. J. Fleisher, E. H. Muller, R. A. Young, R. J. Dineen, J. L. Rich, M. L. Fuller, L. Sirkin, G. C. Wiles, D. L. Pair, D. A. Franzi, J. T. Gurrieri, G. M. Haselton, G. C. Kelley, R. G. LaFleur, and J. S. Street. 1991. Surficial Geologic Map of New York consisting of five sheets: Finger Lakes, Hudson-Mohawk, Niagara, Lower

Hudson and Adirondack. New York State Geological Survey Map and Chart Series. New York State Museum / New York State Geological Survey, Albany.

Caraco, N. F., J. J. Cole, and G. E. Likens. 1989. Evidence for sulfate-controlled phosphorus release from sediments of aquatic systems. Nature 341:316-318.

Chamberlain, T. C. 1890. The method of multiple working hypotheses. Science 15:92-96.

Chambers, R. M. 1997. Porewater chemistry associated with Phragmites and Spartina in a Connecticut tidal marsh. Wetlands 17:360-367.

Crowley, K. and B. Bedford. 2011. Mosses influence phosphorus cycling in rich fens by driving redox conditions in shallow soils. Oecologia 167:253-264.

Dean, W. E. and K. S. Johnson. 1989. Anhydrite Deposits of the United States and Characteristics of Anhydrite Important for Storage of Radioactive Wastes: An inventory of the geologic, physical, hydrologic, and chemical characteristics of anhydrite deposits in the United States and evaluation of anhydrite as a possible medium for the storage of radioactive wastes. USGS Bulletin 1794. United States Government Printing Office, Denver, CO.

Dufey, I., P. Hakizimana, X. Draye, S. Lutts, and P. Bertin. 2009. QTL mapping for biomass and physiological parameters linked to resistance mechanisms to ferrous iron toxicity in rice. Euphytica 167:143-160.

Fisher, D. W., Y. W. Isachsen, and L. V. Rickard. 1970. Geologic Map of New York State, 1970, consisting of 5 sheets: Niagara, Finger Lakes, Hudson-Mohawk, Adirondack, and Lower Hudson. New York State Geological Survey Map and Chart Series. New York State Museum / New York State Geological Survey, Albany.

Geurts, J. J. M., J. M. Sarneel, B. J. C. Willers, J. G. M. Roelofs, J. T. A. Verhoeven, and L. P. M. Lamers. 2009. Interacting effects of sulphate pollution, sulphide toxicity and eutrophication on vegetation development in fens: A mesocosm experiment. Environmental Pollution 157:2072-2081.

Godwin, K. S., J. P. Shallenberger, D. J. Leopold, and B. L. Bedford. 2002. Linking landscape properties to local hydrogeologic gradients and plant species occurrence in minerotrophic fens of New York State, USA: A Hydrogeologic Setting (HGS) framework. Wetlands 22:722-737.

Gross, J., R. J. Stein, A. G. Fett-Neto, and J. P. Fett. 2003. Iron homeostasis related genes in rice. Genetics and Molecular Biology 26:477-497.

Horsak, M., M. Hajek, D. Spitale, P. Hajkova, D. Dite, and J. C. Nekola. 2012. The age of island-like habitats impacts habitat specialist species richness. Ecology 93:1106-1114.

Isachsen, Y. W., E. Landing, J. M. Lauber, L. V. Rickard, and W. B. Rogers. 2000. Geology of New York: a simplified account. Second Edition edition. New York State Museum / Geological Survey, State Education Dept., University of the State of New York, Albany, NY.

Koretsky, C., M. Haveman, L. Beuving, A. Cuellar, T. Shattuck, and M. Wagner. 2007. Spatial variation of redox and trace metal geochemistry in a minerotrophic fen. Biogeochemistry 86:33.

Lamers, L. P. M., H. B. M. Tomassen, and J. G. M. Roelofs. 1998. Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. Environmental Science & Technology 32:199-205.

Lovley, D. R. and E. J. P. Phillips. 1987. Rapid Assay For Microbially Reducible Ferric Iron In Aquatic Sediments. Applied and Environmental Microbiology 53:1536-1540.

Lucassen, E., A. Smolders, L. Lamers, and J. Roelofs. 2005. Water table fluctuations and groundwater supply are important in preventing phosphate-eutrophication in sulphate-rich fens: Consequences for wetland restoration. Plant and Soil 269:109-115.

Lucassen, E., A. J. P. Smolders, and J. G. M. Roelofs. 2002. Potential sensitivity of mires to drought, acidification and mobilisation of heavy metals: the sediment S/(Ca+Mg) ratio as diagnostic tool. Environmental Pollution 120:635-646.

Lucassen, E. C. H. E. T., A. J. P. Smolders, A. L. Van der Salm, and J. G. M. Roelofs. 2004. High groundwater nitrate concentrations inhibit eutrophication of sulphate-rich freshwater wetlands. Biogeochemistry 67:249-267.

McCallan, S. E. A., A. Hartzell, and F. Wilcoxon. 1936. Hydrogen sulphide injury to plants. Contributions from Boyce Thompson Institute 8:189-197.

McCune, B. and J. B. Grace. 2002. Analysis of Ecological Communities. MjM Software Design, Gleneden Beach, OR.

Murphy, J. and J. P. Riley. 1962. A Modified Single Solution Method For Determination Of Phosphate In Natural Waters. Analytica Chimica Acta 26:31-36.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2011. vegan: Community Ecology Package. R package version 2.0-2. http://CRAN.R-project.org/package=vegan.

Olivero, A. M. 2001. Classification and Mapping of New York's Calcareous Fen Communities. New York Natural Heritage Program, Albany, NY.

Qian, P. and J. J. Schoenau. 2002. Practical applications of ion exchange resins in agricultural and environmental soil research. Canadian Journal of Soil Science 82:9-21.

Sanz-Lazaro, C., T. Valdemarsen, A. Marin, and M. Holmer. 2011. Effect of temperature on biogeochemistry of marine organic-enriched systems: implications in a global warming scenario. Ecological Applications 21:2664-2677.

Snowden, R. E. D. and B. D. Wheeler. 1993. Iron Toxicity To Fen Plant-Species. Journal of Ecology 81:35-46.

Stookey, L. L. 1970. Ferrozine - A New Spectrophotometric Reagent For Iron. Analytical Chemistry 42:779-781.

Sutton-Grier, A. E., J. K. Keller, R. Koch, C. Gilmour, and J. P. Megonigal. 2011. Electron donors and acceptors influence anaerobic soil organic matter mineralization in tidal marshes. Soil Biology & Biochemistry 43:1576-1583.

van der Welle, M. E. W., M. Cuppens, L. P. M. Lamers, and T. G. M. Roelofs. 2006. Detoxifying toxicants: Interactions between sulfide and iron toxicity in freshwater wetlands. Environmental Toxicology and Chemistry 25:1592-1597.

Weathers, K. C., M. L. Cadenasso, and S. T. A. Pickett. 2001. Forest edges as nutrient and pollutant concentrators: Potential synergisms between fragmentation, forest canopies, and the atmosphere. Conservation Biology 15:1506-1514.

Weathers, K. C., S. M. Simkin, G. M. Lovett, and S. E. Lindberg. 2006. Empirical modeling of atmospheric deposition in mountainous landscapes. Ecological Applications 16:1590-1607.

Wesley, F. R., S. Gardescu, and P. L. Marks. 2008. Vascular Plant Species of the Cayuga Region of New York State. Cornell University online publications: Species List, Ithaca, NY. 63 pages. http://hdl.handle.net/1813/9413:63.

White, P. J. and M. R. Broadley. 2003. Calcium in plants. Annals of Botany 92:487-511.

Whitmire, S. L. and S. K. Hamilton. 2008. Rates of anaerobic microbial metabolism in wetlands of divergent hydrology on a glacial landscape. Wetlands 28:703-714.

Wieder, R. K., J. B. Yavitt, and G. E. Lang. 1990. Methane production and sulfate reduction in 2 Appalachian peatlands. Biogeochemistry 10:81-104.

Xu, D., J. Xu, Y. He, and P. Huang. 2009. Effect of Iron Plaque Formation on Phosphorus Accumulation and Availability in the Rhizosphere of Wetland Plants. Water, Air, and Soil Pollution 200:79-87.

Zohlen, A. and G. Tyler. 2000. Immobilization of tissue iron on calcareous soil: differences between calcicole and calcifuge plants. Oikos 89:95-106.

CHAPTER 5

SUMMARY AND CONCLUSIONS

The work in this dissertation sought to characterize spatial and temporal heterogeneity in wetland porewater chemistry, especially the reduced sulfur species sulfide, and relate that chemistry to wetland plant community composition. Initially, the goal was to compare the magnitude of influence of sulfur on plant community composition in highly contrasting wetland types ranging from rich fens dominated by groundwater inputs, bogs dominated by precipitation inputs, and marshes dominated by surface water inputs. Subsequently, the scope of the work was narrowed to rich fens only, which encompass a broader range of sulfur inputs than bogs, a narrower range of confounding water table fluctuations than marshes, and a circumneutral pH range where redox-sensitive changes in phosphorus availability linked to sulfide should be most apparent. In addition, rich fens are among the most botanically diverse of all wetlands (Amon et al. 2002), and the as yet incompletely understood controls of this high diversity offered the greatest potential to be explained by direct and indirect effects of sulfide heterogeneity.

Summary

There was a high degree of spatial heterogeneity in rich fen porewater chemistry. Porewater sulfate and calcium were variable but conformance of average concentrations to regional variation in bedrock geology was lower than expected. Sulfide was highly variable within and across fens, ranging over two orders of magnitude in many fens. Fine-scale heterogeneity of sulfide in a single fen (Chapter 4) exceeded the heterogeneity seen in a broader-scale array of regional fens (Chapter 2). Inversely related concentrations of sulfide and ferrous

iron in porewater were consistent with tight chemical coupling but were not readily traceable to phosphorus availability. The fine-scale spatial patterns of sulfide and ferrous iron were conserved across seasons (Chapter 3), making them consistent influences on vegetation. Collectively, the high degree of spatial heterogeneity in sulfide and ferrous iron porewater chemistry, largely undocumented in past freshwater wetland ecology work, underscored the strong potential for there to be a linkage between environmental heterogeneity and plant structure and community composition.

The potential linkage between environmental heterogeneity and plant structure and community composition was in part realized but was not as strong as anticipated. In the intensively sampled fen sulfide reduced total plant cover, reduced cover of the three most frequently occurring species, reduced dicot species density, reduced plant height, and increased bare ground (Chapter 4). Calcium and phosphorus combined with sulfide to explain some plant responses, but phosphorus alone did not explain any plant responses at the fine scale. Sulfide had a more limited influence on vegetation at the regional scale, only secondarily reducing total plant cover and increasing bare ground after first accounting for site-to-site variability (Chapter 2). At the regional scale the influence of porewater chemistry on alpha diversity was ambiguous, but beta diversity declined as a negative power function of sulfide variability. Overall, results from this work confirmed the role of calcium in influencing rich fen vegetation (Clarkson 1965, White and Broadley 2003) and suggested that direct sulfide toxicity was a persistent but more moderate than expected stress to rich fen plants, while indirect sulfide mobilization of phosphorus was less important to plants than sulfide toxicity.

Future research

Future work measuring sulfide and Fe(II) at the sub-cm scale within the plant rhizosphere using diffusive gradients in thin films (DGT) (Devries and Wang 2003), voltammetric microelectrodes (Luther et al. 1998), and other micro-scale methods (Naylor et al. 2006) would help determine the extent to which different plant species influence rates of oxidation of sulfide and Fe(II) in the rhizosphere through variations in radial oxygen loss and root exudates of carbon compounds. Oxidation in the rhizosphere is a key component of sulfide avoidance and any such oxidation in the rhizosphere is a critical complement to internal plant physiological processing of sulfide that is addressed in detail below.

This dissertation identified the net effect of sulfide on vegetation but was not designed to evaluate the relative importance of different mechanisms of sulfide influence on wetland plants. Some plant species may have a high capacity for sulfide avoidance while others may have a high capacity for sulfide tolerance or assimilation. Future work could differentiate sulfide avoidance from sulfide tolerance and sulfide assimilation by looking at the sulfur compounds that have accumulated in plant tissue, as well as the activity of enzymes required for sulfur assimilation. The most common forms of sulfur found in plants are typically free sulfate, the amino acid cysteine (mostly in protein complexes), and glutathione (Rennenberg et al. 2007). Species that exclude sulfide from roots or degas volatile sulfur as H_2S, COS or other gaseous sulfur species from foliar stomata should have limited sulfur accumulation, species that take up sulfide but immediately oxidize it should accumulate sulfate in tissue, and species that take up sulfide and assimilate it should accumulate glutathione. Glutathione is an important reduced form of organic sulfur that can be stored, is readily transported within plants (Bloem et al. 2005) and has roles that range widely from protecting plants from pests, serving as an antioxidant (Bloem et al. 2005, Bloem et al. 2007), and detoxifying xenobiotics and heavy metals (Tausz et al. 2004, Rausch et

al. 2007). Species that assimilate sulfide to cysteine and then glutathione will likely have higher OASTL in the roots than in the shoots. Plant species with the capability to bypass the normal energy-intensive assimilatory sulfate reduction pathway and produce cysteine and glutathione in roots directly from pedospheric H_2S may reap the benefits of glutathione and put on more biomass in controlled experimental conditions than species with low H_2S assimilation capacity. Examining the ratio of reduced glutathione relative to oxidized sulfate in plant tissue of contrasting experimental porewater sulfur treatments would help establish which plant species not only tolerate sulfide but assimilate it, thereby suggesting mechanisms for patterns of species occurrence observed in the field.

Given the paucity of data on freshwater wetland plant responses to sulfide and the sensitivity of the rich fen study sites the descriptive approach employed in this dissertation was appropriate, but to conclusively establish a causative relationship between sulfide and performance of specific plant species future experimental work in a greenhouse would be important. The logical first candidate species for experimental work at range of sulfide concentrations would be those species in which models including sulfide provided the greatest explanatory power in the field. Furthermore, the importance of both presence-absence and quantitative cover components of the models indicates that examination of sulfide in both the establishment and maintenance phases of plant life history is warranted.

Significance

One of the original motivations of this research was to ascertain whether wetlands were losing plant species as a consequence of atmospheric deposition of sulfur, so this question merits being revisited. The research presented in this dissertation was ultimately conducted in rich fens

with geological inputs of sulfur from groundwater for thousands of years that are greater than sulfur inputs from the atmosphere during recent decades, making comparisons difficult. The relatively modest plant response to extremely large variations in sulfide chemistry in rich fens described in this dissertation suggests that plant responses to smaller changes in atmospheric deposition should be even more subtle. Longitudinal studies of wetland plant species composition before and after atmospheric deposition of sulfur would be in the context of a more explicit state of disequilibrium than in the present study focused on long-standing underlying geology, raising the possibility of plant species losses due to *de novo* introduction of sulfur. However, sulfur is present in all living organisms and cycled internally so even bog plant species newly exposed to atmospheric deposition of sulfur have always been exposed to and utilized sulfur. Ultimately, even though it is not possible to extrapolate directly from rich fens with underlying sulfur-rich geology to bogs newly experiencing atmospheric deposition, it currently seems unlikely that atmospheric sulfur deposition has a strong influence on bog plant species composition.

This work may prove to be of greater policy relevance to coastal wetlands than to bogs. Saltwater intrusion is predicted in coastal zones as a consequence of sea level rise. Many coastal wetlands are already low in plant diversity, but a subset of higher diversity near-coastal wetlands with currently stable water table levels and water chemistry in the range seen in freshwater wetlands may soon be inundated with tidal seawater that is rich in sulfate as well as sodium and chloride. Most research will likely focus on overall osmotic stress to plants newly exposed to saltwater, but disentangling the effects of subsequent sulfide accumulation from overall salinity effects may benefit from the approach used in this dissertation to simultaneously address sulfide and more commonly studied calcium. Some plant species may have elevated sensitivity to

sulfide and decline more rapidly than other species in response to inundation by seawater.

The research in this dissertation highlights yet another level of complexity that is not addressed in current federal wetlands policy that aims for achieving no net loss of wetlands area but does not mandate protection of all wetland types. It is already widely recognized in the scientific community that there are very large differences in chemistry and plant community composition among different wetland types. This research adds to the existing literature that addresses further distinctions within wetland types. Specifically, it points to the relevance of considering underlying geology when prioritizing protection or restoration of groundwater-fed ecosystems for maximum protection of biodiversity.

REFERENCES

Amon, J. P., C. A. Thompson, Q. J. Carpenter, and J. Miner. 2002. Temperate zone fens of the glaciated Midwestern USA. Wetlands 22:301-317.

Bloem, E., S. Haneklaus, I. Salac, P. Wickenauser, and E. Schnug. 2007. Facts and fiction about sulfur metabolism in relation to plant-pathogen interactions. Plant Biology 9:596-607.

Bloem, E., S. Haneklaus, and E. Schnug. 2005. Significance of sulfur compounds in the protection of plants against pests and diseases. Journal of Plant Nutrition 28:763-784.

Clarkson, D. T. 1965. Calcium uptake by calcicole and calcifuge Species in the genus *Agrostis* L. Journal of Ecology 53:427-435.

Devries, C. R. and F. Y. Wang. 2003. In situ two-dimensional high-resolution profiling of sulfide in sediment interstitial waters. Environmental Science & Technology 37:792-797.

Luther, G. W., P. J. Brendel, B. L. Lewis, B. Sundby, L. Lefrancois, N. Silverberg, and D. B. Nuzzio. 1998. Simultaneous measurement of O_2, Mn, Fe, I^-, and S(-II) in marine pore waters with a solid-state voltammetric microelectrode. Limnology and Oceanography 43:325-333.

Naylor, C., W. Davison, M. Motelica-Heino, G. A. Van den Berg, and L. M. Van der Heijdt. 2006. Potential kinetic availability of metals in sulphidic freshwater sediments. Science of the Total Environment 357:208-220.

Rausch, T., R. Gromes, V. Liedschulte, I. Muller, J. Bogs, V. Galovic, and A. Wachter. 2007. Novel insight into the regulation of GSH biosynthesis in higher plants. Plant Biology 9:565-572.

Rennenberg, H., C. Herschbach, K. Haberer, and S. Kopriva. 2007. Sulfur Metabolism in Plants: Are Trees Different? Plant Biology 9:620-637.

Tausz, M., H. Sircelj, and D. Grill. 2004. The glutathione system as a stress marker in plant ecophysiology: is a stress-response concept valid? Journal of Experimental Botany 55:1955-1962.

White, P. J. and M. R. Broadley. 2003. Calcium in plants. Annals of Botany 92:487-511.